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L4 ANSWER 1 OF 45 MEDLINE on STN
2004061169. PubMed ID: 14762359. Neonatal vulnerability to ischemia and reperfusion: Cardioplegic arrest causes greater myocardial apoptosis in neonatal lambs than in mature lambs. Karimi Mohsen; Wang Li Xing; Hammel James M; Mascio Christopher E; Abdulhamid Mohamed; Barner Elesa W; Scholz Thomas D; Segar Jeffrey L; Li Wei Gen; Niles Scott D; Caldarone Christopher A. Journal of thoracic and cardiovascular surgery, (2004 Feb) 127 (2) 490-7. Journal code: 0376343. ISSN: 0022-5223. Pub. country: United States. Language: English.

AB OBJECTIVES: Apoptosis is a mechanism for deletion of injured or obsolete cells that is distinct from necrosis and mediated by mitochondrial release of cytochrome c **caspase** activation. Because myocardial apoptosis is a part of normal fetal and postnatal maturation, we hypothesize that neonatal myocardium is more vulnerable to undergo myocardial apoptosis than mature myocardium after cardioplegic arrest. METHODS: Newborn and mature lambs (n = 5 in each group) underwent cardiopulmonary bypass, antegrade crystalloid hyperkalemic cardioplegic arrest for 60 minutes, and a 6-hour recovery period. Myocardium was examined by using terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-digoxigenin nick end labeling (TUNEL), Western

blotting, in vitro kinase assays, and **fluorometric assays** of the activity of **caspases** 3, 8, and 9. Myocardium from nonoperated control subjects (n = 5 in each age group) was also obtained. RESULTS: More TUNEL-positive nuclei were present in the newborn postcardioplegic myocardium (P =.04). **Caspase** 3, 8, and 9 activities were 1.6-fold, 1.5-fold, and 1.4-fold greater in the newborn postcardioplegic myocardium (P =.04, P =.01, and P =.01, respectively). The Bax/Bcl-2 ratio was higher in the newborn postcardioplegic myocardium (P =.04). Apoptosis signal-regulating kinase 1 activity and cleaved **caspase** 3 levels were higher in the newborn postcardioplegic myocardium (P =.02 and P =.009). Mitochondrial release of cytochrome c was greater in the newborn postcardioplegic myocardium (P =.009). CONCLUSIONS: The increased Bax/Bcl-2 ratio in the newborn myocardium suggests a proapoptotic state that is manifested by greater TUNEL staining, cytochrome c release, and cleavage of **caspase** 3. Increased apoptosis signal-regulating kinase 1 activity suggests greater oxidative stress, immature mechanisms to ameliorate oxidative stress, or both in the neonatal myocardium. Mitochondrial release of cytochrome c suggests that apoptosis-related mitochondrial dysfunction might contribute to early postoperative myocardial dysfunction in the neonate.

L4 ANSWER 2 OF 45 MEDLINE on STN DUPLICATE 1

2004050892. PubMed ID: 14751501. Antisense MDM2 sensitizes prostate cancer cells to androgen deprivation, radiation, and the combination. Mu Zhaomei; Hachem Paul; Agrawal Sudhir; Pollack Alan. (Department of Radiation Oncology, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111, USA.) International journal of radiation oncology, biology, physics, (2004 Feb 1) 58 (2) 336-43. Journal code: 7603616. ISSN: 0360-3016. Pub. country: United States. Language: English.

AB PURPOSE: Antisense MDM2 (AS) sensitizes a variety of tumor cell types, including prostate cancer, to radiation and chemotherapy. We have previously described that AS enhances the apoptotic response to androgen deprivation (AD) and that this translates into a reduction in overall cell survival, as measured by clonogenic assay. Because AD + radiation (RT) is a key strategy for the treatment of men with high-risk prostate cancer, AS was tested for the ability to sensitize cells to the combination of AD+RT. METHODS AND MATERIALS: LNCaP cells were cultured in vitro in either complete, androgen deprived (AD), or AD+R1881 (synthetic androgen) medium for 2-3 days before AS was administered. Radiation at 5 Gy was given 18-24 h later. Processing of the cells after RT was done at 3 h for Western blots, 24 and 48 h for trypan blue dye exclusion, 18 h for Annexin V staining by flow cytometric analysis, 18 h for **Caspase** 3+7 quantification by **fluorometric assay**, and immediately for clonogenic survival measured 12-14 days later. There were 18 treatment groups that were studied: lipofectin control, AS, antisense mismatch (ASM), AD, AD+R1881, and RT in all possible combinations. Statistical comparisons between groups were accomplished with one-way analysis of variance using the Bonferroni test, considering all 18 groups. RESULTS: AS caused a reduction in MDM2 expression and an increase in p53 and p21 expression. Early cell death by trypan blue was found to be reflective of the apoptotic results by Annexin V and **Caspase** 3+7. AS caused a significant increase in apoptosis over the lipofectin control, AD, and RT controls. Apoptosis was further increased significantly by the addition of AD or RT to AS. When AS, AD, and RT were combined, there was a consistent increase in early cell death over AS+AD and AS+RT by all of the assay methods, although this increase was not significant. Overall cell death measured by clonogenic assay revealed synergistic cell killing of AS+RT beyond that of ASM+RT and RT alone, and AS+RT+AD beyond that of AS+RT, AS+RT+AD+R1881, ASM+RT+AD, and ASM+RT+AD+R1881. CONCLUSION: AS sensitizes cells to AD, RT, and AD+RT and shows promise in the treatment of the full range of patients with prostate cancer. AS has the potential to sensitize the primary tumor to AD+RT and

metastasis to AD.

L4 ANSWER 3 OF 45 MEDLINE on STN DUPLICATE 2
2003491826. PubMed ID: 14568554. Rapid annexin-V labeling in synaptosomes. Gylys Karen H; Fein Jeffrey A; Wiley Dorothy J; Cole Gregory M. (UCLA School of Nursing and Brain Research Institute, Box 956919, Factor Building, Los Angeles, CA 90095, USA.. kgylys@sonnet.ucla.edu) . Neurochemistry international, (2004 Feb) 44 (3) 125-31. Journal code: 8006959. ISSN: 0197-0186. Pub. country: England: United Kingdom. Language: English.

AB Distal neuronal terminals may be the site of apoptotic events and early synapse loss in neurodegenerative disease. To examine apoptosis in synaptic regions, we established a cell-free assay using a rat brain crude synaptosomal preparation (P-2 fraction) as a model system. The apoptosis marker annexin-V was used to measure phosphatidylserine (PS) exposure, and to ensure that only intact terminals were assayed, synaptosomes were dual labeled with a viability marker (calcein AM). Fluorescence was quantified by flow cytometry analysis. Annexin-V labeling increased rapidly in synaptosomes, following a 1 min incubation with staurosporine. However, increased **caspase**-3-like activity was not measured until 30 min with a **fluorometric assay**. The addition of a peptide inhibitor of **caspase**-3-like activity (Ac-DEVD-CHO) during homogenization was not able to block the initial increase in annexin labeling, but resulted in a partial blockade of annexin labeling after 30 min. These data demonstrate that PS externalization and **caspase** activation occur rapidly in this widely used neurochemical preparation.

L4 ANSWER 4 OF 45 MEDLINE on STN
2004080374. PubMed ID: 14970883. Caffeic acid (CA) protects cerebellar granule neurons (CGNs) from apoptosis induced by neurotoxin 1-methyl-4-phenylpyridnium (MPP(+)). Tian Xue Fei; Pu Xiao Ping. (Department of Molecular and Celluar Pharmacology, Peking University School of Pharmaceutical Sciences, Beijing 100083, China.) Beijing da xue xue bao. Yi xue ban = Journal of Peking University. Health sciences, (2004 Feb 18) 36 (1) 27-30. Journal code: 101125284. ISSN: 1671-167X. Pub. country: China. Language: Chinese.

AB OBJECTIVE: To assess the effects of caffeic acid (CA) on MPP(+)-induced cerebellar granule neurons (CGNs) apoptosis. METHODS: CGNs were pretreated with caffeic acid at 55, 110 and 220 micromol/L for 6 h, then treated with 100 micromol/L MPP(+) for 24 h (concentration-effect relationship). In addition CGNs were pretreated with caffeic acid at 110 micromol/L for 0 h, 6 h, 12 h, and 24 h, respectively, then treated with 100 micromol/L MPP(+) for 24 h (time-response relationship). Besides, after treatment with MPP(+) for 24 h, CGNs were incubated with caffeic acid at 55, 110 and 220 micromol/L,respectively. Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and **caspase**-3 activity was assayed by **caspase**-3 **fluorometric assay** kit. RESULTS: MTT assay revealed that caffeic acid significantly inhibited cell viability decrease induced by MPP(+), and **caspase**-3 **fluorometric assay** showed that caffeic acid efficiently suppressed **caspase**-3 activation in CGNs induced by MPP(+). CONCLUSION: Caffeic acid (CA) can significantly protect CGNs from apoptosis induced by MPP(+) and may provide a useful therapeutic strategy for the treatment of Parkinson's disease.

L4 ANSWER 5 OF 45 MEDLINE on STN DUPLICATE 3
2003362292. PubMed ID: 12894543. Analysis of apoptosis signaling pathway in human cancer cells by codeinone, a synthetic derivative of codeine. Hitosugi Naoko; Nagasaka Hiroshi; Sakagami Hiroshi; Matsumoto Isao; Kawase Masami. (Department of Anesthesiology, Saitama Medical School, Department of Dental Pharmacology, Meikai University School of Dentistry, Saitama,

Japan.. nao-hito@rc4.so-net.ne.jp) . Anticancer research, (2003 May-Jun) 23 (3B) 2569-76. Journal code: 8102988. ISSN: 0250-7005. Pub. country: Greece. Language: English.

AB We have recently found that codeinone, an oxidation metabolite of codeine, induced apoptosis, characterized by internucleosomal DNA fragmentation and mitochondrial cytochrome c release in HL-60 human promyelocytic leukemic cell lines, most effectively among 10 opioids. These findings prompted us to investigate whether codeinone induces apoptosis in other human cancer cells and possible changes in mitochondrial enzyme. FACS analysis demonstrated that codeinone induced the production of ANNEXIN-positive apoptotic cells in three different human cancer cells (HL-60, MCF7, A549). The apoptotic cells were visualized by microscopical observation after staining with Hoechst (H)-33342. **Fluorometric assay** showed that codeinone time-dependently activated **caspase** 3 and **caspase** 9, but not **caspase** 8, suggesting the activation of intrinsic apoptotic signaling pathway via mitochondria. Western blot analysis demonstrated that codeinone enhanced the Pro-apoptotic Bax protein expression, but reduced the anti-apoptotic Bcl-2 protein expression. Codeinone did not significantly change the manganese superoxide dismutase (MnSOD) activity nor its mRNA expression. This apoptosis-inducing activity, in conjunction with antinociceptive activity, further substantiated the antitumor potential of codeinone.

L4 ANSWER 6 OF 45 MEDLINE on STN DUPLICATE 4

2003126407. PubMed ID: 12639677. Role of mitochondrial cytochrome c in cocaine-induced apoptosis in rat testes. Li Haikun; Xu Liping; Dunbar Joseph C; Dhabuwala C B. (Department of Urology, Wayne State University School of Medicine, Detroit, Michigan 48201, USA.) Urology, (2003 Mar) 61 (3) 646-50. Journal code: 0366151. ISSN: 1527-9995. Pub. country: United States. Language: English.

AB OBJECTIVES: We have previously demonstrated that cocaine exposure leads to apoptosis in rat testes. To understand further the mechanism of cocaine-induced testicular damage, we studied the effect of cocaine on cytochrome c release from the mitochondria. We also determined the **caspase** 3, **caspase** 8, and **caspase** 9 activities in rat testes after chronic cocaine exposure. METHODS: Thirty-day-old male Sprague-Dawley rats received cocaine hydrochloride or equal volumes of normal saline subcutaneously daily for 90 days. The testes were removed at 15, 30, and 90 days of cocaine or saline administration. Mitochondria and cytosolic fractions from testes were isolated. Western blotting was performed in both fractions using anti-cytochrome c antibody. **Caspase** 3, **caspase** 8, and **caspase** 9 activities were determined by **fluorometric assay**. RESULTS: The expression of cytochrome c protein in the cytosolic fraction was increased on day 15 and persisted for up to 90 days after cocaine injection compared with controls. However, the expression of cytochrome c in testes was decreased in the mitochondria fraction on days 15, 30, and 90 after cocaine injections compared with the corresponding controls. The **caspase** activity study showed **caspase** 3 and **caspase** 9 activities increased in cocaine-treated testes at each point of the study compared with the corresponding controls. However, the **caspase** 8 activity in cocaine-treated testes did not change significantly at each point of the study compared with the corresponding controls. CONCLUSIONS: Our results suggest that the release of cytochrome c from mitochondria and its subsequent activation of **caspase** 9 and **caspase** 3 in testes play a key role in cocaine-induced germ cell apoptosis. Our findings also indicate that cocaine-induced testicular germ cell apoptosis in rats is at least initiated through a mitochondria-associated pathway.

L4 ANSWER 7 OF 45 MEDLINE on STN DUPLICATE 5

2003167089 Document Number: 22571380. PubMed ID: 12683923. Increased

TNF-alpha expression in cultured mouse embryos exposed to teratogenic concentrations of glucose. Torchinsky A; Brokhman I; Shepshelovich J; Orenstein H; Savion S; Zaslavsky Z; Koifman M; Dierenfeld H; Fein A; Toder V. (Department of Embryology and Teratology, Sackler School of Medicine, Tel-Aviv University, Israel.. arkadyt@post.tau.ac.il) . Reproduction, (2003 Apr) 125 (4) 527-34. Journal code: 100966036. ISSN: 1470-1626. Pub. country: England: United Kingdom. Language: English.

AB Diabetes-induced early embryonic death is accompanied by an increased expression of tumour necrosis factor alpha (TNF-alpha) in the embryonic microenvironment. The aim of the present study was to evaluate whether diabetes-induced embryopathic stress may also alter the expression of TNF-alpha produced by the embryo itself. As a model, whole postimplantation embryos were cultured for 24 h in a medium with high concentrations of glucose, one of the main diabetes-associated teratogenic metabolites. An anomaly such as an open neural tube was used as an end-point characterizing the glucose-induced teratogenic effect and the number of somites was counted to evaluate growth retardation induced by glucose. The expression of TNF-alpha (by immunohistochemistry), apoptosis (by TdT-mediated dUTP nick-end labelling; TUNEL) and the activity of **caspases** 3 and 8 (by a **fluorometric assay**) were evaluated in normal and malformed embryos. Ninety-seven per cent of the embryos exposed to 1300 mg glucose dl(-1) exhibited an open neural tube. The percentage of malformed embryos was smaller in media containing 800 and 500 mg glucose dl(-1) (68 and 37%, respectively) but it still exceeded significantly the value registered in embryos developing in a normoglycaemic medium (12%). In addition, a significant decrease in the number of somites was observed in embryos developing in media containing 1300 and 800 mg glucose dl(-1). Malformed embryos exhibited a greater number of nuclei that were positive in the TUNEL assay as well as a higher amount of active **caspase** 8 compared with normal embryos (with closed neural folds). TNF-alpha expression was detected in the neuroepithelial layer of the neural tube of the malformed embryos, whereas the expression of this cytokine was weak, if detectable, in normal embryos. Together, these findings indicate that TNF-alpha produced by the embryo may be involved in regulating the response of embryos to diabetes-generated embryopathic stress.

L4 ANSWER 8 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

2004:93444 Document No.: PREV200400086636. Role of ERK activation in cisplatin induced **caspase** activation and apoptosis in LLC-PK1 cells. Jo, Sang-Kyung [Reprint Author]; Sung, Suah [Reprint Author]; Cho, Won Yong [Reprint Author]; Kim, Hyoung Kyu [Reprint Author]; Won, Nam Hee. Division of Nephrology, Department of Internal Medicine, Korea University Hospital, Seoul, South Korea. Journal of the American Society of Nephrology, (November 2003) Vol. 14, No. Abstracts Issue, pp. 274A. print. Meeting Info.: Meeting of the American Society of Nephrology Renal Week. San Diego, CA, USA. November 12-17, 2003. American Society of Nephrology. CODEN: JASNEU. ISSN: 1046-6673. Language: English.

L4 ANSWER 9 OF 45 MEDLINE on STN DUPLICATE 6

2003010110 Document Number: 22387660. PubMed ID: 12500091. Increased S-nitrosothiols and S-nitrosoalbumin in cerebrospinal fluid after severe traumatic brain injury in infants and children: indirect association with intracranial pressure. Bayir Hulya; Kochanek Patrick M; Liu Shang-Xi; Arroyo Antonio; Osipov Anatoly; Jiang Jianfei; Wisniewski Stephan; Adelson P David; Graham Steven H; Kagan Valerian E. (Safar Center for Resuscitation Research, University of Pittsburgh Medical Center, Department of Critical Care Medicine, University of Pittsburgh School of Medicine, Pennsylvania 15213, USA.) JOURNAL OF CEREBRAL BLOOD FLOW AND METABOLISM, (2003 Jan) 23 (1) 51-61. Journal code: 8112566. ISSN: 0271-678X. Pub. country: United States. Language: English.

AB Nitric oxide (NO) is implicated in both secondary damage and recovery

after traumatic brain injury (TBI). Transfer of NO groups to cysteine sulfhydryls on proteins produces S-nitrosothiols (RSNO). S-nitrosothiols may be neuroprotective after TBI by nitrosylation of N-methyl-D-aspartate receptor and **caspases**. S-nitrosothiols release NO on decomposition for which endogenous reductants (i.e., ascorbate) are essential, and ascorbate is depleted in cerebrospinal fluid (CSF) after pediatric TBI. This study examined the presence and decomposition of RSNO in CSF and the association between CSF RSNO level and physiologic parameters after severe TBI. Cerebrospinal fluid samples (n = 72) were obtained from 18 infants and children on days 1 to 3 after severe TBI (Glasgow Coma Scale score < 8) and 18 controls. Cerebrospinal fluid RSNO levels assessed by **fluorometric assay** peaked on day 3 versus control (1.42 +/- 0.11 micromol/L vs. 0.86 +/- 0.04, P< 0.05). S-nitrosoalbumin levels were also higher after TBI (n = 8, 0.99 +/- 0.09 micromol/L on day 3 vs. n = 6, 0.42 +/- 0.02 in controls, P< 0.05). S-nitrosoalbumin decomposition was decreased after TBI. Multivariate analysis showed an inverse relation between CSF RSNO and intracranial pressure and a direct relation with barbiturate treatment. Using a novel assay, the presence of RSNO and S-nitrosoalbumin in human CSF, an approximately 1.7-fold increase after TBI, and an association with low intracranial pressure are reported, supporting a possible neuroprotective role for RSNO. The increase in RSNO may result from increased NO production and/or decreased RSNO decomposition.

L4 ANSWER 10 OF 45 MEDLINE on STN DUPLICATE 7

2002383655 Document Number: 22127225. PubMed ID: 12131680. Role of anti-tumor necrosis factor-alpha in ischemia/reperfusion injury in isolated rat liver in a blood-free environment. Ben-Ari Ziv; Hochhauser Edith; Burstein Idan; Papo Orit; Kaganovsky Ella; Krasnov Tatyana; Vamichkim Alexey; Vidne Bernardo A. (Liver Institute and Department of Medicine D, Rabin Medical Center, Beilinson Campus, Petah Tiqva, Israel.) TRANSPLANTATION, (2002 Jun 27) 73 (12) 1875-80. Journal code: 0132144. ISSN: 0041-1337. Pub. country: United States. Language: English.

AB BACKGROUND: Warm ischemia/reperfusion injury during liver transplantation is the most important cause of primary nonfunction of liver allografts. Tumor-necrosis factor (TNF)-alpha apparently mediates tissue damage by inducing apoptosis and/or necrosis in liver transplants. The aim of the study was to determine, using an isolated rat liver model, if pretreatment with anti-TNF-alpha monoclonal antibodies can attenuate ischemia/reperfusion liver injury. Specifically, its effect on liver cell apoptosis through the modulation of **caspase** activity was examined in a blood-free environment. METHODS: Isolated rat livers were perfused with Krebs-Henseleit solution and randomly divided into three groups: (1) continuous perfusion for 165 min (control); (2) perfusion for 90 min, break for 60 min (ischemia), and reperfusion for 15 min; (3) as with group 2, but with administration of monoclonal mouse anti-rat TNF-alpha monoclonal antibodies before induction of ischemia. **Caspase**-3- and -9-like activity was measured by **fluorometric assay**, and apoptotic cells were identified by morphological criteria and application of the terminal deoxnucleotidyl transferase-mediated dUTP nick-end-labeling (Tunel) assay. RESULTS: Portal pressure increased significantly in group 2 (14.8+/-2.3 mm Hg) compared to group 3, which showed no change (P<0.05). Significant amounts of TNF-alpha were detected in the effluent in group 2 at 1 min of reperfusion (147+/-8.9 pg/ml) compared to group 3 (30+/-6.7 pg/ml, P<0.05). Statistically significant reductions in liver enzyme levels were also noted in the animals pretreated with TNF-alpha antibodies (P<0.02). **Caspase**-3 and -9 activity was significantly decreased (270 and 160%, respectively) in group 3 compared to group 2 (P<0.005 and <0.05, respectively). A significant reduction in postischemic hepatic injury was noted on Tunel assay: many apoptotic hepatocyte cells were detected in group 2 but not in livers pretreated with monoclonal mouse anti-TNF-alpha

antibodies (group 3). CONCLUSIONS: Neutralization with specific monoclonal antibodies against TNF before ischemia induction can attenuate postischemic hepatic injury. Apoptotic injury seems to be ameliorated through modulation of **caspase**-3- and -9-like activity.

L4 ANSWER 11 OF 45 MEDLINE on STN DUPLICATE 8
2002245049 Document Number: 21979334. PubMed ID: 11984520. Critical role of **caspases** in the regulation of apoptosis and proliferation of mucosal T cells. Sturm Andreas; Mohr Susanne; Fiocchi Claudio. (Division of Gastroenterology, Department of Medicine, University Hospital of Cleveland, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106-4952, USA.) GASTROENTEROLOGY, (2002 May) 122 (5) 1334-45. Journal code: 0374630. ISSN: 0016-5085. Pub. country: United States. Language: English.

AB BACKGROUND & AIMS: **Caspases** are critical mediators of apoptosis and proliferation of peripheral blood T cells (PBT), but their role in lamina propria T cells (LPT), a cell population highly susceptible to apoptosis, has not been explored. METHODS: RA(+), RO(+) PBT, and LPT were activated with CD3, CD2, and CD28 antibodies, and **caspase** activity, apoptosis, and proliferation were measured by a **fluorometric assay**, DNA content, and thymidine incorporation, respectively. Levels of FLIP, an endogenous inhibitor of **caspase** 8, were measured by immunoblotting. RESULTS: In RA(+) and RO(+) PBT, activation leads to significant increase of **caspase** activity but not cell death, whereas in LPT a lower elevation of **caspase** activity was followed by a marked degree of apoptosis. Based on the results of its inhibition, **caspase** 8 seemed to be essential for LPT apoptosis but, in contrast to RA(+) PBT, had no effect on proliferation. In addition, compatible with their differential susceptibility to apoptosis, levels of FLIP were lower in LPT than PBT. CONCLUSIONS: The high susceptibility of LPT to apoptosis is associated with a distinct regulation of **caspase** 8 activity, which seems to reflect their mucosal origin rather than simply their memory status. This unique behavior may allow proper control of mucosal T-cell proliferation while still permitting elimination by apoptosis in the face of excessive antigenic pressure.

L4 ANSWER 12 OF 45 MEDLINE on STN DUPLICATE 9
2002690793 Document Number: 22339243. PubMed ID: 12451262. 17beta-Estradiol protects isolated human pancreatic islets against proinflammatory cytokine-induced cell death: molecular mechanisms and islet functionality. Contreras Juan L; Smyth Cheryl A; Bilbao Guadalupe; Young Carlton J; Thompson J Anthony; Eckhoff Devin E. (Transplant Center, University of Alabama at Birmingham, Birmingham, AL, USA.. Juan.Contreras@ccc.uab.edu) . TRANSPLANTATION, (2002 Nov 15) 74 (9) 1252-9. Journal code: 0132144. ISSN: 0041-1337. Pub. country: United States. Language: English.

AB INTRODUCTION: Proinflammatory cytokines (PIC) (interleukin-1beta, interferon-gamma, and tumor necrosis factor alpha) are released after intraportal islet transplantation lead to functional suppression and islet apoptosis. Estradiol has been shown to promote survival of cells undergoing PIC-induced apoptosis. In this study, we evaluated the effects of estradiol on isolated human pancreatic islet (IHPI) survival after exposure to PIC and analyzed potential mechanisms of action. METHODS: Hand-picked, freshly isolated IHPI were incubated with PIC and estradiol. Viability was analyzed from single islet cells stained with ethidium bromide and acridine orange, apoptosis using a quantitative kit, NF-kappaB nuclear translocation using a promoter-Luciferase NF-kappaB responsive construct, mitochondrial permeability transition using the ApoAlert Mitochondrial kit, and **caspase** 9 by a **fluorometric assay**. In vitro functionality was examined by static incubation, and a limited number of islets were transplanted in nonobese diabetic,

severe combined immunodeficient mice. RESULTS: 17beta-Estradiol induced a dose-dependent increase in islet viability, an effect partially reversed by the estrogen receptor antagonist ICI 182,780. In vitro, islets treated with estradiol presented higher stimulation index. Euglycemia was achieved in 6 of 12 animals that received estradiol-treated islets compared with 1 of 12 control animals. Lower NF-kappaB nuclear translocation, cytochrome release, and **caspase** 9 activation occurred in islets treated with estradiol. CONCLUSIONS: Estradiol promoted IHPI survival and improved functionality after PIC exposure in vitro and in vivo after transplantation. The molecular mechanisms involved included a decrease in NF-kappaB nuclear translocation, decrease in mitochondrial cytochrome release, and **caspase** 9 activation. The use of estradiol might be beneficial in clinical islet transplantation.

L4 ANSWER 13 OF 45 MEDLINE on STN DUPLICATE 10

2002717368. PubMed ID: 12479703. **Caspase** activation is required for gemcitabine activity in multiple myeloma cell lines. Nabhan Chadi; Gajria Devika; Krett Nancy L; Gandhi Varsha; Ghias Kulsoom; Rosen Steven T. (Division of Hematology and Oncology, Department of Medicine, Robert H. Lurie Comprehensive Cancer Center, Feinberg School of Medicine, Northwestern University Medical School, Chicago, Illinois 60611, USA.) Molecular cancer therapeutics, (2002 Nov) 1 (13) 1221-7. Journal code: 101132535. ISSN: 1535-7163. Pub. country: United States. Language: English.

AB The objective of this study was to determine potential mechanisms of apoptotic activity of gemcitabine, a pyrimidine nucleoside analogue, in the MM1.S multiple myeloma (MM) cell line. A MM cell line that is sensitive to glucocorticoids (MM1.S) was used for this study. Immunoblotting analysis, cell cycle assays, and annexin V staining were performed to determine whether gemcitabine induced apoptosis in this model. Furthermore, we attempted to delineate the apoptotic pathway by measuring **caspase**-8 and -9 activity using **fluorometric assays**. Loss of mitochondrial membrane potential was measured by flow cytometry. Gemcitabine treatment caused apoptosis in MM cell lines as measured by an increase in DNA cleavage, an increase in annexin V binding, a decrease in the mitochondrial membrane potential, and activation of **caspase** activity. Furthermore, cleavage of the **caspase** substrate poly(ADP-ribose) polymerase and **caspase** -3 activation were documented as early as 8 h after treatment with gemcitabine. **Caspase**-8 and -9 were activated by gemcitabine treatment in this cell line, suggesting several mechanisms of action including death receptor pathway and mitochondrial damage. The addition of interleukin 6 to MM1.S cells treated with gemcitabine offered no protection against gemcitabine-induced cell death. Gemcitabine induced apoptosis in the MM1.S cell line, and its activity required **caspase** activation. There is a suggestion that mitochondrial integrity is being affected with gemcitabine in this system. Gemcitabine acts independently of interleukin 6, suggesting potential important therapeutic implications in MM patients.

L4 ANSWER 14 OF 45 MEDLINE on STN DUPLICATE 11

2002679569. PubMed ID: 12438947. Simvastatin induces activation of the serine-threonine protein kinase AKT and increases survival of isolated human pancreatic islets. Contreras Juan L; Smyth Cheryl A; Bilbao Guadalupe; Young Carlton J; Thompson J Anthony; Eckhoff Devin E. (Transplant Center, Division of Transplantation, University of Alabama at Birmingham, 748 Lyons-Harrison Research Building, 701 19th Street South, Birmingham, AL 35294, USA.. juan.contreras@ccc.uab.edu) . Transplantation, (2002 Oct 27) 74 (8) 1063-9. Journal code: 0132144. ISSN: 0041-1337. Pub. country: United States. Language: English.

AB BACKGROUND: Pancreatic islets are susceptible to myriad insults that occur

during islet isolation and transplantation. Studies demonstrated the role of Akt in regulating pancreatic beta-cell growth and survival. Activation of Akt maintains Bad phosphorylation and prevents its binding to mitochondrial targets, decreases **caspase**-9 activity, and prevents the translocation of forkhead transcription factors (FKHR). Simvastatin activates Akt in mammalian cells; therefore, we investigated the role of simvastatin on human pancreatic islets (HPI) survival. METHODS: HPI were treated with simvastatin, with and without LY294002, an inhibitor of phosphoinositide 3-kinase. PI viability was examined with ethidium bromide-acridine orange, and apoptosis was examined using a quantitative assay. Akt, Bad, FKHR phosphorylation, and mitochondrial cytochrome release were analyzed by Western blots. **Caspase**-9 activity was assessed by a **fluorometric assay**. A limited number of HPI were transplanted after simvastatin treatment in diabetic NOD-SCID mice. RESULTS: Low levels of Akt phosphorylation (activation) were demonstrated early after islet isolation. Akt activation; increase in islet viability; and decrease in Bad phosphorylation, cytochrome release, **caspase**-9 activation, and translocation of FKHR were observed after simvastatin treatment, effects reversed by LY294002. Among recipients of islets without simvastatin, none demonstrated reversal of diabetes after the transplant. In contrast, 58% of the recipients given islets treated with simvastatin remained euglycemic 30 days after the transplant. CONCLUSIONS: Targeting the survival pathway with simvastatin exerts a cytoprotective effect on isolated PI. Activation of the Akt pathway is a potential new therapeutic approach to reduce loss of functional islet mass to bolster success in clinical islet transplantation.

L4 ANSWER 15 OF 45 MEDLINE on STN DUPLICATE 12
2002441466 Document Number: 22188038. PubMed ID: 12199150.
Caspase inhibition protects nerve terminals from in vitro degradation. Gylys Karen H; Fein Jeffrey A; Cole Gregory M. (UCLA School of Nursing, Brain Research Institute, Box 956919, Los Angeles, California 90095, USA.. kgvlys@sonnet.ucla.edu) . NEUROCHEMICAL RESEARCH, (2002 Jun) 27 (6) 465-72. Journal code: 7613461. ISSN: 0364-3190. Pub. country: United States. Language: English.

AB **Caspase** activation and apoptotic events may take place in terminal regions far removed from the cell body and contribute to synapse loss in neurodegenerative diseases. For examination of events in terminals, we have developed a cell-free assay using quantitative flow cytometric analysis (fluorescence-activated cell sorting) of neuronal particles in a P2 synaptosomal preparation (P-2) from rat brain as a model system. Staurosporine-induced loss of neuronal particles was blocked by nonselective **caspase** inhibition (z-VAD-fmk) and by calpain inhibition (calpain inhibitor II [ALLM]). Phosphatidylserine exposure was increased in the P-2 by staurosporine treatment, and this increase was blocked by a peptide inhibitor of **caspase**-3-like activity (Ac-DEVD-CHO). Increased **caspase** activity in the crude synaptosomal fraction was confirmed by direct measurement with a **fluorometric assay**. These results indicate activation of both **caspase** and calpain in the P-2 fraction and suggest a role for these cysteine proteases in the in vitro degradation of nerve terminals.

L4 ANSWER 16 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2002:323619 Document No.: PREV200200323619. Impact of polyunsaturated fatty acid supplementation on apoptosis in intestinal epithelial cells. Lu, Jing [Reprint author]; Adler, Luba [Reprint author]; Caplan, Michael S.; Jilling, Tamas. Pediatrics, Evanston Northwestern Healthcare, 2650 Ridge Ave, Evanston, IL, 60201, USA. FASEB Journal, (March 20, 2002) Vol. 16, No. 4, pp. A261. print.
Meeting Info.: Annual Meeting of the Professional Research Scientists on

Experimental Biology. New Orleans, Louisiana, USA. April 20-24, 2002. CODEN: FAJOEC. ISSN: 0892-6638. Language: English.

AB We have previously shown that polyunsaturated fatty acid (PUFA) supplementation reduced the incidence of experimental necrotizing enterocolitis (NEC) in a neonatal rat model. In this model platelet-activating factor (PAF) plays a critical role, and the incidence of NEC correlates with increased apoptosis of epithelial cells. Herein, we examined the effect of arachidonic acid (AA) and/or docosahexaenoic acid (DHA) supplementation on apoptosis in intestinal epithelial cells (IEC-6) with, or without administration of PAF. **Caspase** (Casp) activity was measured using **fluorometric assays** and DNA fragmentation was evaluated by ELISA of cytoplasmic histone DNA complexes. Supplementation of AA (100 muM), DHA (67 muM), and AA+DHA (100 muM+67muM) was significantly associated with 54+-14%, 65+-9% and 76+-7% reducion of Casp 3 activity compared to untreated control, respectively, whereas DNA fragmentation remained similar. In the absence of PUFA, PAF stimulated Casp 3 by 168+-18%, Casp 9 by 174+-21% and DNA fragmentation by 268+-58%. PUFA supplementation completely abrogated the induction of **caspase** activity and DNA fragmentation by PAF. We speculate that the protective role of PUFA on experimental NEC may be due to its ability to block the induction of apoptosis by PAF.

L4 ANSWER 17 OF 45 MEDLINE on STN DUPLICATE 13

2003010278 Document Number: 22404342. PubMed ID: 12516634. The role of pro- and anti-apoptotic molecular interactions in embryonic maldevelopment. Toder V; Carp H; Fein A; Torchinsky A. (Department of Embryology and Teratology, Sackler School of Medicine, Tel-Aviv University, Ramat Aviv, Tel-Aviv, Israel.. toder@post.tau.ac.il) . AMERICAN JOURNAL OF REPRODUCTIVE IMMUNOLOGY, (2002 Oct) 48 (4) 235-44. Ref: 99. Journal code: 8912860. ISSN: 1046-7408. Pub. country: Denmark. Language: English.

AB PROBLEM: Pregnancy loss and the occurrence of inborn structural anomalies are often preceded by excessive apoptosis in targeted embryonic and extraembryonic tissues. Apoptogenic stimuli activate both death and survival, signaling cascades consisting of molecules acting as activators and effectors, or negative regulators of apoptosis. The interplay between these cascades determines whether the cell which is exposed to an apoptogenic stimulus dies or survives. This review summarizes the functioning of pro- and anti-apoptotic molecules in embryos responding to various teratogens. The effect of potentiation of the maternal immune system on these molecules is also discussed. METHODS OF STUDY: The data on the functioning of various pro- and anti-apoptotic molecules in embryos exposed to various developmental toxicants, and embryos developing in a diabetic environment are reviewed. Techniques such as the TUNEL method, DNA fragmentation assay, electromobility shift assay (EMSA), **fluorometric assay**, immunohistochemistry, Western blot, In situ hybridization, have been used in our studies to detect apoptosis, and evaluate the functioning of molecules such as TNFalpha, **caspases**, NF-kappaB and IkappaB, p53, and bcl-2 in different embryonic and extraembryonic tissues. RESULTS: Our and other data summarized in this review have demonstrated that the doses of developmental toxicants required to induce pregnancy loss and gross structural anomalies induce excessive apoptosis shortly after treatment. Depending on the intensity and type of targeted tissues, this apoptosis was accompanied by alterations in the activity of the molecules which act as activators and effectors (e.g. **caspase** 3, **caspase** 8, **caspase** 2, p53) or negative regulators (bcl-2, NF-kappaB) of apoptosis. Maternal immunopotentiation, which decreases the level of induced and spontaneous pregnancy loss and the incidence and severity of teratogen-induced structural anomalies has been shown to modulate the expression of these molecules both in embryonic tissues and at the feto-maternal interface. CONCLUSIONS: The data presented in this review

suggest that molecules such as TNFalpha, **caspase** 3, **caspase** 8, NF-kappaB, p53 and bcl-2, which are involved in the regulation of apoptosis, may also be involved in determining the sensitivity of the embryo to developmental toxicants. Maternal immunopotentiation may modulate the functioning of these molecules.

L4 ANSWER 18 OF 45 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2002:783789 The Genuine Article (R) Number: 596VP. The role of pro- and anti-apoptotic molecular interactions in embryonic maldevelopment. Toder V (Reprint); Carp H; Fein A; Torchinsky A. Tel Aviv Univ, Dept Embryol & Teratol, Sackler Sch Med, IL-69978 Tel Aviv, Israel (Reprint). AMERICAN JOURNAL OF REPRODUCTIVE IMMUNOLOGY (OCT 2002) Vol. 48, No. 4, pp. 235-244. Publisher: BLACKWELL MUNKSGAARD. 35 NORRE SOGADE, PO BOX 2148, DK-1016 COPENHAGEN, DENMARK. ISSN: 8755-8920. Pub. country: Israel. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB PROBLEM: Pregnancy loss and the occurrence of inborn structural anomalies are often preceded by excessive apoptosis in targeted embryonic and extraembryonic tissues. Apoptogenic stimuli activate both death and survival, signaling cascades consisting of molecules acting as activators and effectors, or negative regulators of apoptosis. The interplay between these cascades determines whether the cell which is exposed to an apoptogenic stimulus dies or survives. This review summarizes the functioning of pro- and anti-apoptotic molecules in embryos responding to various teratogens. The effect of potentiation of the maternal immune system on these molecules is also discussed.

METHODS OF STUDY: The data on the functioning of various pro- and antiapoptotic molecules in embryos exposed to various developmental toxicants, and embryos developing in a diabetic environment are reviewed. Techniques such as the TUNEL method, DNA fragmentation assay, electromobility shift assay (EMSA), **fluorometric assay**, immunohistochemistry, Western blot, In situ hybridization, have been used in our studies to detect apoptosis, and evaluate the functioning of molecules such as TNFalpha, **caspases**, NF-kappaB and IkappaB, p53, and bcl-2 in different embryonic and extraembryonic tissues.

RESULTS: Our and other data summarized in this review have demonstrated that the doses of developmental toxicants required to induce pregnancy loss and gross structural anomalies induce excessive apoptosis shortly after treatment. Depending on the intensity and type of targeted tissues, this apoptosis was accompanied by alterations in the activity of the molecules which act as activators and effectors (e.g. **caspase** 3, **caspase** 8, **caspase** 2, p53) or negative regulators (bcl-2, NF-kappaB) of apoptosis. Maternal immunopotentiation, which decreases the level of induced and spontaneous pregnancy loss and the incidence and severity of teratogen-induced structural anomalies has been shown to modulate the expression of these molecules. both in embryonic tissues and at the feto-maternal interface.

CONCLUSIONS: The data presented in this review suggest that molecules such as TNFalpha, **caspase** 3, **caspase** 8, NF-kappaB, p53 and bcl-2, which are involved in the regulation of apoptosis, may also be involved in determining the sensitivity of the embryo to developmental toxicants. Maternal immunopotentiation may modulate the functioning of these molecules.

L4 ANSWER 19 OF 45 MEDLINE on STN DUPLICATE 14
2002236858 Document Number: 21923105. PubMed ID: 11925595.
JTE-522-induced apoptosis in human gastric adenocarcinoma [correction of adenocarcinoma] cell line AGS cells by **caspase** activation accompanying cytochrome C release, membrane translocation of Bax and loss of mitochondrial membrane potential. Li Hong-Liang; Chen Dan-Dan; Li Xiao-Hong; Zhang Hai-Wei; Lu Jun-Hua; Ren Xian-Da; Wang Cun-Chuan. (Department of Pharmacology, Jinan University Pharmacy College, Guangzhou

510632, Guangdong Province, China.. tsam@jnu.edu.cn) . World J Gastroenterol, (2002 Apr) 8 (2) 217-23. Journal code: 100883448. ISSN: 1007-9327. Pub. country: China. Language: English.

AB AIM: To investigate the role of the mitochondrial pathway in JTE-522-induced apoptosis and to investigate the relationship between cytochrome C release, **caspase** activity and loss of mitochondrial membrane potential (Deltapsim). METHODS: Cell culture, cell counting, ELISA assay, TUNEL, flow cytometry, Western blot and **fluorometric assay** were employed to investigate the effect of JTE-522 on cell proliferation and apoptosis in AGS cells and related molecular mechanism. RESULTS: JTE-522 inhibited the growth of AGS cells and induced the apoptosis. **Caspases** 8 and 9 were activated during apoptosis as judged by the appearance of cleavage products from procaspase and the **caspase** activities to cleave specific fluorogenic substrates. To elucidate whether the activation of **caspases** 8 and 9 was required for the apoptosis induction, we examined the effect of **caspase**-specific inhibitors on apoptosis. The results showed that **caspase** inhibitors significantly inhibited the apoptosis induced by JTE-522. In addition, the membrane translocation of Bax and cytosolic release of cytochrome C accompanying with the decrease of the uptake of Rhodamin 123, were detected at an early stage of apoptosis. Furthermore, Bax translocation, cytochrome C release, and **caspase** 9 activation were blocked by Z-VAD.fmk and Z-IETD-CHO. CONCLUSION: The present data indicate a crucial association between activation of **caspases** 8, 9, cytochrome C release, membrane translocation of Bax, loss of Deltapsim and JTE-522-induced apoptosis in AGS cells.

L4 ANSWER 20 OF 45 MEDLINE on STN DUPLICATE 15

2002050070 Document Number: 21634541. PubMed ID: 11772970. Activation of **caspases** in intestinal villus epithelial cells of normal and nematode infected rats. Hyoh Y; Ishizaka S; Horii T; Fujiwara A; Tegoshi T; Yamada M; Arizono N. (Department of Medical Zoology, Kyoto Prefectural University of Medicine, Kyoto, Japan.) GUT, (2002 Jan) 50 (1) 71-7. Journal code: 2985108R. ISSN: 0017-5749. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: Small intestinal epithelial cells (IEC) show apoptosis in physiological turnover of cells and in certain inflammatory diseases. AIMS: To investigate the role of **caspases** in the progression of IEC apoptosis in vivo. METHODS: IEC were separated along the villus-crypt axis from the jejunum of normal and Nippostrongylus brasiliensis infected rats at 4 degrees C. **Caspases** were examined by a **fluorometric assay** method, histochemistry, and immunoblotting. RESULTS: Villus cell rich IEC from normal rats exhibited a high level of **caspase**-3-like activity whereas activities of **caspase**-1, -8, and -9 were negligible. Immunoblotting analysis of villus cell rich IEC revealed partial cleavage of procaspase-3 into a 17 kDa molecule as well as cleavage of a **caspase**-3 substrate, poly(ADP-ribose) polymerase (PARP), whereas in crypt cell rich IEC, **caspase**-3 cleavage was less significant. **Caspase**-3 activity was also observed histochemically in villus epithelium on frozen sections of the normal small intestine. IEC prepared at 4 degrees C did not reveal nuclear degradation whereas subsequent incubation in a suspension at 37 degrees C induced intense nuclear degradation within one hour in accordance with increases in active **caspase**-3. This apoptosis was partially suppressed by the **caspase** inhibitor Z-VAD-fmk. Nematode infected animals showed villus atrophy together with significant increases in levels of **caspase**-3 in IEC but not of **caspase**-1, -8, or -9. CONCLUSION: **Caspase**-3 may have an important role in the physiological replacement of IEC as well as in progression of IEC apoptosis induced by nematode infection.

L4 ANSWER 21 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

2003:357241 Document No.: PREV200300357241. Troxatyl and STI571 Combination Therapy for Chronic Myeloid Leukemia: Preclinical In Vitro and In Vivo Evaluation. Orsolic, Nada [Reprint Author]; Giles, Francis [Reprint Author]; Beran, Miloslav [Reprint Author]; Cortes, Jorge [Reprint Author]; Albitar, Maher [Reprint Author]; Kantarjian, Hagop [Reprint Author]; Verstovsek, Srdan [Reprint Author]. Leukemia, The University of Texas, MD Anderson Cancer Center, Houston, TX, USA. Blood, (November 16 2002) Vol. 100, No. 11, pp. Abstract No. 3107. print.
Meeting Info.: 44th Annual Meeting of the American Society of Hematology. Philadelphia, PA, USA. December 06-10, 2002. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB Troxatyl triphosphate (converted by the intracellular phosphorylation of Troxatyl) is a potent inhibitor and chain terminator for human cellular DNA polymerases and has a unique pattern of cellular uptake and metabolism. On a Phase I study, Troxatyl had significant antileukemia activity in patients with refractory disease. (Giles et al, JCO: 19:762:2001). In a subsequent Phase II study, 6 patients with chronic myeloid leukemia (CML) in blastic phase of 16 evaluable (37%) achieved a return to chronic phase disease (Giles et al, JCO: 20:656, 2002). In the present preclinical study we have evaluated in vitro and in vivo activity of Troxatyl, alone and in combination with STI571, against human CML cell lines sensitive and resistant to STI571. Cell lines included KBM5 and KBM7 cells that differ in their inherent sensitivity to STI571, the number of copies of the BCR/ABL fusion gene, and their response to STI571 exposure (G0/G1 cell cycle arrest in KBM5 vs. apoptosis in KBM7). We developed STI resistant sublines, KBM5-R and KBM7-R, by culturing the cells with increasing concentrations of STI571; both achieved IC50 twenty times higher then that in parental line. In 3 day MTS assay all 4 cell lines showed similar sensitivity to treatment with Troxatyl (IC50 = 0.5 to 1 muM). Combined treatment with Troxatyl and STI571 revealed additive or synergistic effect. Greater apoptotic response (assessed by **fluorometric assay** for **caspase**-3/7 activity)
was seen with combined treatment then with either agent alone in KBM-7 and KBM-7R cells. In 8-day clonogenic assay Troxatyl showed significant activity against CML progenitors (IC50 = 0.01 muM) from patients both sensitive (i.e. untreated) and resistant to STI571. However, we found no selectivity of Troxatyl against malignant cells as progenitors from normal donors were affected to the same extent. Animal model for in vivo experiments was SCID mouse bearing KBM5 or KBM5-R cells; without therapy mice die by day 33. Troxatyl was administered i.p. daily x5 days starting on Day 20, at doses of 5, 10, 20, or 25 mg/kg. STI571 was administered i.p. twice a day x10 days at the dose of 50 mg/kg starting on day 25. In this setting of late stage disease treatment with Troxatyl showed significant activity in prolonging survival while STI571 did not; combined therapy, however, suggested synergistic activity. Final results of the in vivo experiments with Troxatyl and STI571 will be presented. Our findings indicate that Troxatyl has significant activity in late stage CML and that combining it with STI571 is a very reasonable clinical approach.

L4 ANSWER 22 OF 45 MEDLINE on STN DUPLICATE 16

2001429649. PubMed ID: 11382748. Ionizing radiation-induced apoptosis in ataxia-telangiectasia fibroblasts. Roles of **caspase**-9 and cellular inhibitor of apoptosis protein-1. Zhang Y; Dimtchev A; Dritschilo A; Jung M. (Department of Radiation Medicine, Division of Radiation Research, Vincent T. Lombardi Cancer Center, Georgetown University School of Medicine, Washington, D. C. 20007, USA.) Journal of biological chemistry, (2001 Aug 3) 276 (31) 28842-8. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Ionizing radiation (IR) has been shown to induce apoptosis to a greater extent in a fibroblast cell line AT5BIVA derived from an individual with ataxia-telangiectasia (AT) than in control fibroblasts. However, the

signaling pathway that underlies IR-induced apoptosis in AT cells has remained unknown. The mechanism of apoptosis in response to gamma-irradiation has now been examined in three AT fibroblast lines (AT3BIVA, AT4BIVA, and AT5BIVA) derived from different individuals with AT. The apoptotic indexes of these cell lines at 72 h after irradiation were 12, 31, and 35%, respectively, compared with a value of 2.3% for control fibroblasts. Immunoblot analysis and **fluorometric assays** revealed that the extents of IR-induced activation of **caspase**-3 and **caspase**-9 were markedly greater in AT4BIVA and AT5BIVA cells than in AT3BIVA and control cells. Furthermore, the basal abundance of the apoptotic inhibitor, a cellular inhibitor of apoptosis proteins (c-IAP-1), was markedly reduced in AT4BIVA and AT5BIVA cells compared with that in AT3BIVA and control cells. The overexpression of either **caspase**-9 mutant forms or recombinant c-IAP-1 in AT5BIVA cells inhibited the IR-induced activation of **caspases**-3 and 9 and reduced the apoptotic index of the irradiated cells. These results indicate that the extent of IR-induced apoptosis in different AT cell lines is inversely related to the abundance of c-IAP-1 and directly related to the extent of activation of **caspases**-3 and 9.

L4 ANSWER 23 OF 45 MEDLINE on STN DUPLICATE 17
2001428350. PubMed ID: 11477563. Cisplatin-induced apoptosis of mesothelioma cells is affected by potassium ion flux modulator amphotericin B and bumetanide. Marklund L; Henriksson R; Grankvist K. (Department of Clinical Chemistry, Umea University, Umea, Sweden.) International journal of cancer. Journal international du cancer, (2001 Aug 15) 93 (4) 577-83. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.

AB Chemotherapeutic anti-cancer drugs induce cell death by the process of apoptosis. Efflux of potassium ions (K(+)) is necessary for cell volume reduction during apoptosis and increased inward pumping of K(+) thus counteracts apoptosis. Potassium flux modulation could therefore interact with apoptosis and affect the efficiency of cancer chemotherapeutics. We explored if the K(+) efflux stimulator amphotericin B, with or without the Na(+), K(+), 2Cl(-)-cotransport (K(+) influx) blocker bumetanide, could affect cisplatin- and carboplatin-induced apoptosis and cytotoxicity in the pulmonary mesothelioma cell line (P31). Apoptosis was determined by quantifying free nucleosomes and **caspase**-3 activity, and cytotoxicity was determined by clone formation and a **fluorometric assay**. The pan-**caspase** enzyme inhibitor Boc-D-FMK was used to further determine the role of **caspase** activity in K(+)-flux-modulated cisplatin-/carboplatin-induced apoptosis and cytotoxicity. Amphotericin B (3.2 micromol/L) combined with bumetanide (100 micromol/L) potentiated cisplatin-induced free nucleosome and **caspase**-3 activity. The combination of the K(+) modulators did not, however, increase cisplatin cytotoxicity. The **caspase** inhibitor Boc-D-FMK, but unexpectedly also bumetanide, markedly reduced cisplatin cytotoxicity and annihilated the augmented cytotoxicity of cisplatin in the presence of amphotericin B. Carboplatin cytotoxicity was reduced by bumetanide, but not affected by amphotericin B. Carboplatin and carboplatin/bumetanide cytotoxicity was further reduced by Boc-D-FMK. We conclude that the ability of cisplatin, and to a lesser extent carboplatin, to induce apoptosis is indeed influenced by cellular potassium flux modulators. We suggest that K(+) ionophores such as amphotericin B, and K(+) influx blockers such as bumetanide, alone or in combination, should be further evaluated for their potential clinical usefulness in influencing tumor cell apoptosis induced by cisplatin and other cancer chemotherapeutics.

L4 ANSWER 24 OF 45 BIOSIS on STN
2002:152831 Document No.: PREV200200152831. Molecular mechanisms on low rate

of apoptosis with MS-5 during ex-vivo expansion of human cord blood CD34+ cells. Lee, Kyoung-Eun [Reprint author]; Yoo, Eun-Sun; Lee, Mi-Ae; Ahn, Jee-Young [Reprint author]; Im, Seock-Ah [Reprint author]; Lee, Sun-Mee [Reprint author]; Lee, Seung-Joo; Lee, Soon-Nam [Reprint author]; Chung, Wha-Soon; Seong, Chu-Myong [Reprint author]. Hematology, Ewha Women's University, Seoul, South Korea. Blood, (November 16, 2001) Vol. 98, No. 11 Part 2, pp. 339b. print.
Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 2. Orlando, Florida, USA. December 07-11, 2001. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB While, several investigators have reported the success of allogeneic cord blood transplantation even in regular size adults using ex-vivo expansion in stroma-free liquid culture, the long term engraftment ability of expanded human cord blood(HCB) CD34+ cells is controversial. Recently, we have found the different patterns of apoptosis according to cytokines(ie, FL, TPO, SCF) during ex-vivo expansion without the feeder layer with HCB(Br. J. Hematology; 1999:107:176-185). However, the molecular mechanisms of apoptosis including **caspase** cascade activaiton and Fas-Mediated pathway with or without the feeder layer(MS-5) on apoptosis during expansion of HCB CD34+ cells were unknown. In the present study, CD34+ cells isolated from HCB were cultured with MS-5 feeder layer or in a stroma-free liquid culture system with thrombopoietin(TPO), flt3-ligand(FL) and stem cell factor(SCF). During the culture, for up to 3 weeks, apoptosis was measured by staining 7-amino-actinomycin D(7AAD) along the concurrent immonophenotying of CD34+ with flow cytometry. Activities of **caspase** 8 and **caspase** 9 were determined by using a **fluorometric assay** kt(R and D Systems, Inc., Minneapolis, MN). Ribonuclease protection assay was performed on total RNA to see the changes of moleculars on apoptosis. The results are as follows; 1)The percentage of apoptotic and dead cell fractions during ex-vivo expansion of HCB CD34+ cells in the presence or absence of MS-5 are 3.8% on day 0, 9.1% vs 20.2% on day 7, 11.9% vs 21.8% on day 14 and 10.8% vs 23.7% on day 21 respectively. 2)the fluoresence intensity(FI) of **caspase** 9 during ex-vivo expansion with MS-5 was decreased in half rather than without MS-5 on day 7, 14, 21 respectively. The FI of **caspase** 8 also showed similar patterns as **caspase** 9. 3)Lower mRNA of FAS, FADD, FLICE, TRAIL and TNRp55 were found by ribonuclease protection assay in ex-vivo expansion with MS-5. In summary, the percentage of apoptotic and dead cell fraction along with the pattern of **caspase** 8 and 9 activity during ex-vivo expansion of HCB CD34+ cells in the presence of MS-5 was lower than ex-vivo expansion in absence of MS-5. The Fas-mediated pathway seems to be one fo the molecular mechanisms on apoptosis. Further studies to understand on apoptosis will be necessary in applying for effective ex-vivo HCB expansion.

L4 ANSWER 25 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2001:290081 Document No.: PREV200100290081. Isoelectric focusing and enzyme overlay membrane analysis of **caspase** 3 activation. Breithaupt, Thomas B. [Reprint author]; Shires, Adam L.; Voehringer, David W.; Herzenberg, Leonard A.; Herzenberg, Leonore A.. Department of Biochemistry, Des Moines University-Osteopathic Medical Center, 3200 Grand Avenue, Des Moines, IA, 50312, USA. Thomas.Breithaupt@dmu.edu. Analytical Biochemistry, (May 15, 2001) Vol. 292, No. 2, pp. 313-316. print.
CODEN: ANBCA2. ISSN: 0003-2697. Language: English.

L4 ANSWER 26 OF 45 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2001:976015 The Genuine Article (R) Number: 498FX. Detection of a decrease in green fluorescent protein fluorescence for the monitoring of cell death: An assay amenable to high-throughput screening technologies. Steff A M; Fortin M; Arguin C; Hugo P (Reprint). PROCREA BioSci, Div Res & Dev, 6100

Royalmt, Montreal, PQ H4P 2R2, Canada (Reprint); PROCREA BioSci, Div Res & Dev, Montreal, PQ H4P 2R2, Canada. CYTOMETRY (1 DEC 2001) Vol. 45, No. 4, pp. 237-243. Publisher: WILEY-LISS. DIV JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012 USA. ISSN: 0196-4763. Pub. country: Canada. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background: Reliable assessment of cell death is now pivotal to many, research programs aiming at generating new anti-tumor compounds or at screening cDNA libraries. Stich approaches need to rely on reproducible, easy-to-handle, and rapid microplate-based cytotoxicity assays that are an-tenable to high-throughput screening (HTS) technologies. We describe it method for the direct measurement of cell death, based on the detection of a decrease in fluorescence observed following death induction in cells expressing enhanced green fluorescent protein (EGFP).

Methods: Cell death was induced by it variety of apoptotic stimuli in various EGFP-expressing mammalian cell lines, including those routinely used in anti-cancer drug screening. Decrease in fluorescence was assessed either by flow cytometry (and compared with other apoptotic markers) or by a fluorescence microplate reader.

Results: Cells expressing EGFP exhibited a decrease in fluorescence when treated by various agents, such as chemotherapeutic drugs, UV irradiation, or **caspase**-independent cell death inducers. Kinetics and sensitivity of this EGFP-based assay were comparable to those of traditional apoptosis markers such as annexin-V binding, pro. gen species pidium iodide incorporation, or reactive oxygen production. We also show that the decrease in EGFP fluorescence is directly quantifiable in a fluorescence-based microplate assay. Furthermore, analysis of EGFP protein content in cells undergoing cell death demonstrates that the decrease in fluorescence does not arise from degradation of the protein.

Conclusions: This novel GFP-based microplate assay combines sensitivity and rapidity, is easily amenable to HTS Setups, making it an assay of choice for cytotoxicity evaluation. (C) 2001 Wiley Liss, Inc.

L4 ANSWER 27 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN

2000:861787 Document No. 134:38853 A **fluorometric assay** for **caspase** activity that uses fusion proteins of green fluorescent protein that exhibit fluorescent resonance energy transfer. Xanthoudakis, Steven; Tawa, Paul; Cassady, Robin; Nicholson, Donald (Merck Frosst Canada and Co., Can.). PCT Int. Appl. WO 2000073437 A1 20001207, 50 pp. DESIGNATED STATES: W: AU, CA, JP, US, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-CA620 20000525. PRIORITY: US 1999-PV136286 19990527.

AB Provided are substrates and assays for the identification of **caspase** activators and inhibitors. The substrates are fusion proteins comprising two green fluorescent proteins (GFPs) with a linker peptide comprising at least one **caspase** cleavage site. The intact fusion protein exhibits fluorescent resonance energy transfer (FRET) between the GFPs. Following **caspase** cleavage of the linker peptide, the two GFPs become separated and FRET is diminished. The method can be adapted to high throughput assays. Sequences claimed in the document were not published with it.

L4 ANSWER 28 OF 45 MEDLINE on STN DUPLICATE 18

2000486397. PubMed ID: 11036845. The inhibition of spontaneous and tumor necrosis factor-alpha induced neutrophil apoptosis by crystals of calcium pyrophosphate dihydrate and monosodium urate monohydrate. Tudan C; Fong D; Duronio V; Burt H M; Jackson J K. (Faculty of Pharmaceutical Sciences and the Department of Medicine, University of British Columbia, Vancouver, Canada.) Journal of rheumatology, (2000 Oct) 27 (10) 2463-72. Journal code: 7501984. ISSN: 0315-162X. Pub. country: Canada. Language: English.

AB OBJECTIVE: Spontaneous neutrophil apoptosis may be inhibited by various proinflammatory stimuli. which may result in prolonged lifetimes and responses of these phagocytic cells with the potential for extended inflammation. We investigated the effect of short term incubation of opsonized crystals of monosodium urate monohydrate (MSUM) or calcium pyrophosphate dihydrate (CPPD) on both spontaneous and tumor necrosis factor-alpha (TNF-alpha) induced neutrophil apoptosis. METHODS: Peripheral neutrophils were incubated with plasma opsonized crystals of CPPD or MSUM in the presence or absence of TNF-alpha for 4 h at 37 degrees C. Apoptosis was determined using 3 separate assays: (1) an agarose DNA fragmentation assay, (2) a cytoplasmic histone associated DNA fragmentation assay, and (3) a **caspase** 3 **fluorometric assay**. RESULTS: All 3 assays showed similar results. Both MSUM and CPPD crystals inhibited spontaneous apoptosis in neutrophils. TNF-alpha induced high levels of apoptosis in neutrophils. However, co-incubation of the cells with TNF-alpha and crystals resulted in the inhibition of apoptosis to levels below those of control cells. Pretreatment of neutrophils with the protein synthesis inhibitor cycloheximide prevented the inhibition of apoptosis by crystals. CONCLUSION: These data support the concept of crystal induced inhibition of neutrophil apoptosis as part of the pathophysiology of the diseases collectively known as crystal induced arthritis.

L4 ANSWER 29 OF 45 CAPLUS COPYRIGHT 2004 ACS on STN

2000:656273 Document No. 133:251002 Role of CD95 and **caspase** activity for endotoxin-associated hepatotoxicity and lethality. Wanner, G. A.; Mica, L.; Hentze, H.; Kunstle, G.; Kolb, S.; Trentz, O.; Ertel, W. (Klinik fur Unfallchirurgie, Universitatsspital Zurich, Switz.). Chirurgisches Forum fuer Experimentelle und Klinische Forschung 509-512 (German) 2000. CODEN: CFEKA7. ISSN: 0303-6227. Publisher: Springer-Verlag.

AB The aim of this study was to analyze the role of **caspase** activity and CD95 for endotoxin-mediated hepatic microvascular injury and endotoxin-associated lethality. C3H/HeN mice were i.v. administered LPS (E. coli; 10 mg/kg b.w.) in the presence or absence of the **caspase** inhibitor z-VAD-fmk or a neutralizing CD95 fusion protein (CD95-Fp). Control animals received saline. After 6 h animals (n = 6/group) underwent laparotomy under rompun/ketanest anesthesia and hepatic microcirculation was analyzed using intravital fluorescence microscopy, including quant. anal. of sinusoidal perfusion and leukocyte adherence in postsinusoidal venules. Liver injury was assessed by measuring plasma AST and ALT levels. **Caspase**-1-like and -3-like activities were measured using specific **fluorometric assays**. Finally, a survival study was performed, comparing LPS-treated mice with mice that received z-VAD-fmk (n = 9/group). Hepatic microcirculation after LPS administration was characterized by severe sinusoidal perfusion failure and increased adherence of leukocytes to the venular wall at 6 h. Repetitive administration of z-VAD-fmk inhibited sinusoidal perfusion failure and attenuated leukocyte accumulation in postsinusoidal venules. LPS-induced increase of liver enzymes was decreased by z-VAD-fmk. Neutralization of CD95 had no influence on any of these parameters. **Caspase**-3-like activities were comparable in all groups. In contrast, LPS induced an increase of **caspase**-1-like activity in liver tissue which was blocked by z-VAD-fmk but not by CD95-Fp. All animals of the LPS group died within 24 h while 7 out of 9 animals survived this time period after z-VAD-fmk treatment. These data indicate that CD95-independent activation of **caspases** is a key event in LPS-associated hepatotoxicity. **Caspase** inhibition may represent a new therapeutic concept to counteract endotoxin-mediated liver injury and lethality.

L4 ANSWER 30 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

2001:308979 Document No.: PREV200100308979. Arsenic trioxide induces apoptosis in HTLV-I infected T-cell lines and fresh adult T-cell leukemia cells through **caspase**-dependent pathway. Ishitsuka, Kenji; Ikeda, Ryuji; Utsunomiya, Atae [Reprint author]; Uozumi, Kimiharu [Reprint author]; Hanada, Shuichi [Reprint author]; Suzuki, Shinsuke [Reprint author]; Takeuchi, Shogo [Reprint author]; Takatsuka, Yoshifusa [Reprint author]; Takeshita, Taketsugu [Reprint author]; Ohno, Nobuhito [Reprint author]; Arima, Terukatsu [Reprint author]. 2nd Department of Internal Medicine, Kagoshima University, Kagoshima, Japan. Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 341a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology. San Francisco, California, USA. December 01-05, 2000. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB We have reported that arsenic trioxide (As2O3) induces apoptosis in HTLV-I infected T-cell lines and fresh ATL cells. The present study aimed to clarify the pathway of apoptosis in HTLV-I infected T-cell lines, MT-1 and MT-2, and fresh ATL cells separated from peripheral blood of patients with acute and chronic type ATL. Cells were treated up to 72 hours at clinically tolerable As2O3 concentrations (1-2 muM) shown to be safe in patients with acute promyelocytic leukemia. **Caspase** activity was determined by **fluorometric assays**. N-benzyloxycarbonyl-Val-Ala-DL-Asp-fluoromethyl ketone (z-VAD, Bachem) and N-benzyloxycarbonyl-Asp-Glu-Val-Asp-fluoromethyl ketone (z-DEVD, Kamiya biomedical) were used as broad-spectrum **caspase** inhibitor and **caspase** 3 inhibitor, respectively. ZB4 (MBL), CD95 receptor-blocking antibody, was used to determine whether As2O3 induces CD95-dependent apoptosis. Numbers of sub-G1 (apoptotic) cells were determined by flow cytometry using a DNA prep reagent kit (Coulter). Changes of mitochondrial transmembrane potential during apoptosis were detected by an Apoalert mitochondrial membrane sensor kit (Clontech). Cleavage of poly (adenosine diphosphate-ribose) polymerase (PARP) was assessed by Western blot analysis using nuclear protein extracted from the cells. **Caspase** 3, 8 and 9 were significantly activated at a dose dependent manner. The cultivation time to acquire maximum activation of these enzymes varied between 24 hours and 72 hours in 5 cases of fresh ATL cells. Loss of mitochondrial transmembrane potential was observed at a time and dose dependent manner. Cleavage of PARP was shown during As2O3 treatment. Furthermore, prior exposure to z-VAD and z-DEVD at the concentration of 40 muM completely inhibited apoptosis induced by 2 muM of As2O3 in MT-1 and MT-2 cells. In fresh ATL cells, at the same concentration of z-VAD completely inhibited apoptosis, but z-DEVD did lesser extent. While pretreatment with ZB4 did not show such inhibitions in these cells. In conclusion, As2O3-induced apoptotic pathway in these HTLV-I infected T-cell lines and fresh ATL cells is **caspase** -dependent and is associated with mitochondrial events, followed by cleavage of PARP. In addition, the apoptosis induced by As2O3 is independent of CD95 ligand/receptor interaction.

L4 ANSWER 31 OF 45 MEDLINE on STN DUPLICATE 19

2000408552 Document Number: 20363485. PubMed ID: 10903590. Protective effects of ischemic preconditioning for liver resection performed under inflow occlusion in humans. Clavien P A; Yadav S; Sindram D; Bentley R C. (Division of Transplantation and Hepatobiliary Surgery, and the Department of Visceral and Transplantation Surgery, Zurich University Medical Center, Switzerland.) ANNALS OF SURGERY, (2000 Aug) 232 (2) 155-62. Journal code: 0372354. ISSN: 0003-4932. Pub. country: United States. Language: English.

AB OBJECTIVE: To determine whether ischemic preconditioning protects the human liver against a subsequent period of ischemia in patients undergoing hemihepatectomy, and to identify possible underlying protective mechanisms of ischemic preconditioning, such as inhibition of hepatocellular

apoptosis. SUMMARY BACKGROUND DATA: Ischemic preconditioning is a short period of ischemia followed by a brief period of reperfusion before a sustained ischemic insult. Recent studies in rodents suggest that ischemic preconditioning is a simple and powerful protective modality against ischemic injury of the liver. The underlying mechanisms are thought to be related to downregulation of the apoptotic pathway. METHODS: Twenty-four patients undergoing hemihepatectomy for various reasons alternatively received ischemic preconditioning (10 minutes of ischemia and 10 minutes of reperfusion) before transection of the liver performed under inflow occlusion for exactly 30 minutes. Liver wedge and Tru-cut biopsy samples were obtained at the opening of the abdomen and 30 minutes after the end of the hepatectomy. Serum levels of aspartate transferase, alanine transferase, bilirubin and prothrombin time were determined daily until discharge. Hepatocellular apoptosis was evaluated by in situ terminal deoxynucleotidyl transferase mediated d-UTP nick end-labeling (TUNEL) assay and electron microscopy. **Caspase** 3 and 8 activities were measured in tissue using specific **fluorometric assays**. RESULTS: Serum levels of aspartate transferase and alanine transferase were reduced by more than twofold in patients subjected to ischemic preconditioning versus controls. The analysis of a subgroup of patients with mild to moderate steatosis indicated possible increased protective effects of ischemic preconditioning. In situ TUNEL staining demonstrated a dramatic reduction in the number of apoptotic sinusoidal lining cells in the ischemic preconditioning group. Electron microscopy confirmed features of apoptosis present in control but not in ischemic preconditioning patients. There was no significant difference in **caspase** 3 and 8 activity when patients with ischemic preconditioning were compared with controls. CONCLUSIONS: Ischemic preconditioning is a simple and effective modality protecting the liver against subsequent prolonged periods of ischemia. This strategy may be a more attractive technique than intermittent inflow occlusion, which is associated with increased blood loss during each period of reperfusion.

L4 ANSWER 32 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2001:9226 Document No.: PREV200100009226. Shiga toxin-induced apoptosis via a **caspase**-3-dependent pathway: Prevention by brefeldin A. Kojio, S. [Reprint author]; Zhang, H. M. [Reprint author]; Ohmura, M. [Reprint author]; Gondaira, F. [Reprint author]; Kobayashi, N.; Yamamoto, T. [Reprint author]. Sch. of Med., Niigata Univ., Niigata, Japan. Abstracts of the Interscience Conference on Antimicrobial Agents and Chemotherapy, (2000) Vol. 40, pp. 42. print.
Meeting Info.: 40th Interscience Conference on Antimicrobial Agents and Chemotherapy. Toronto, Ontario, Canada. September 17-20, 2000. Interscience Conference on Antimicrobial Agents and Chemotherapy; American Society of Microbiology.
Language: English.

L4 ANSWER 33 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2001:135006 Document No.: PREV200100135006. Differential activation of **caspases** after traumatic spinal cord injury in the rat. Huang, X. [Reprint author]; VanGelderen, J.; Calva-Cerqueira, D.; Reed, J. C.; Krajewski, S.; Faden, A. I.; Knoblach, S. M.. Georgetown University, Washington, DC, USA. Society for Neuroscience Abstracts, (2000) Vol. 26, No. 1-2, pp. Abstract No.-863.11. print.
Meeting Info.: 30th Annual Meeting of the Society of Neuroscience. New Orleans, LA, USA. November 04-09, 2000. Society for Neuroscience.
ISSN: 0190-5295. Language: English.
AB Apoptotic cell death programs are induced in response to CNS injury. Activation of **caspase** enzymes plays an important role in the evolution of such programs. Multiple **caspases** have been identified, yet relative roles of the majority of these in spinal cord

injury (SCI) are unknown. To address this issue, we examined activation of **caspases** 1, 3, 8 and 9 at 1, 4, 24, 72 and 168 hr after moderate SCI induced by the weight drop method. **Caspases** exist as proenzymes that are activated upon proteolytic cleavage. Therefore, homogenates from the injury site were assessed by Western blotting methods utilizing antibodies for specific activated **caspase** subunits. Two different antibodies detected 12 and 17 kDa active subunits of **caspase** 3 respectively, from 1 to 72 hr after injury. Twelve and 15 kDa cleavage fragments of **caspase** 9 were detected at these times, as well. Antibodies for active subunits of **caspase** 1 or 8 did not detect cleaved forms of these enzymes at any time after SCI. Results from **fluorometric assays** of **caspase** 1 and 3 activity supported those from Western blot analysis. Double-label immunohistochemistry utilizing antibodies for cell-type specific markers and active forms of **caspases** 3, 8 and 9 revealed their presence in neurons and glia at 4 and 72 hr after injury. **Caspase** 8 positive cells were less abundant than either **caspase** 9 or 3 positive cells. These data indicate that **caspases** are selectively activated from hours to several days after SCI and suggest that **caspase** 9 and 3 pathways play major roles in SCI-induced apoptotic programs. Supported by CRPF KA1-9905-2 to SMK and DOD 17-93-V-3018 to AIF.

L4 ANSWER 34 OF 45 MEDLINE on STN DUPLICATE 20
2000042113. PubMed ID: 10576694. Fluorescent molecular probes V: a sensitive **caspase**-3 substrate for **fluorometric assays**. Liu J; Bhalgat M; Zhang C; Diwu Z; Hoyland B; Klaubert D H. (Molecular Probes, Inc., Eugene, OR 97402, USA.) Bioorganic & medicinal chemistry letters, (1999 Nov 15) 9 (22) 3231-6. Journal code: 9107377. ISSN: 0960-894X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB (Z-Asp-Glu-Val-Asp)-Rhodamine 110 [(Z-DEVD)2-Rh 110] was prepared and characterized as a sensitive fluorogenic substrate for the determination of **caspase**-3 activity.

L4 ANSWER 35 OF 45 MEDLINE on STN DUPLICATE 21
2000004612. PubMed ID: 10534344. Ischemic preconditioning protects the mouse liver by inhibition of apoptosis through a **caspase**-dependent pathway. Yadav S S; Sindram D; Perry D K; Clavien P A. (Hepatobiliary and Transplant Laboratory, Department of Surgery, Duke University Medical Center, Durham, NC 27710, USA.) Hepatology (Baltimore, Md.), (1999 Nov) 30 (5) 1223-31. Journal code: 8302946. ISSN: 0270-9139. Pub. country: United States. Language: English.

AB A short period of ischemia and reperfusion, called ischemic preconditioning, protects various tissues against subsequent sustained ischemic insults. We previously showed that apoptosis of hepatocytes and sinusoidal endothelial cells is a critical mechanism of injury in the ischemic liver. Because **caspases**, calpains, and Bcl-2 have a pivotal role in the regulation of apoptosis, we hypothesized that ischemic preconditioning protects by inhibition of apoptosis through down-regulation of **caspase** and calpain activities and up-regulation of Bcl-2. A preconditioning period of 10 minutes of ischemia followed by 15 minutes of reperfusion maximally protected livers subjected to prolonged ischemia. After reperfusion, serum aspartate transaminase (AST) levels were reduced up to 3-fold in preconditioned animals. All animals subjected to 75 minutes of ischemia died, whereas all those who received ischemic preconditioning survived. Apoptosis of hepatocytes and sinusoidal endothelial cells, assessed by in situ TUNEL assay and DNA fragmentation by gel electrophoresis, was dramatically reduced with preconditioning. **Caspase** activity, measured by poly (adenosine diphosphate ribose) polymerase (PARP) proteolysis and a specific **caspase**-3 **fluorometric assay**, was

inhibited by ischemic preconditioning. The antiapoptotic mechanism did not involve calpain-like activity or Bcl-2 expression because levels were similar in control and preconditioned livers. In conclusion, ischemic preconditioning confers dramatic protection against prolonged ischemia via inhibition of apoptosis through down-regulation of **caspase** 3 activity, independent of calpain-like activity or Bcl-2 expression.

L4 ANSWER 36 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
2000:18627 Document No.: PREV200000018627. Ischemic preconditioning protects the mouse liver by inhibition of apoptosis through a **caspase** -dependent pathway. Yadav, Surinder S.; Sindram, David; Perry, David K.; Clavien, Pierre-Alain [Reprint author]. Duke University Medical Center, Durham, NC, 27710, USA. Hepatology, (Nov., 1999) Vol. 30, No. 6, pp. 1223-1231. print.
CODEN: HPTLD9. ISSN: 0270-9139. Language: English.

AB A short period of ischemia and reperfusion, called ischemic preconditioning, protects various tissues against subsequent sustained ischemic insults. We previously showed that apoptosis of hepatocytes and sinusoidal endothelial cells is a critical mechanism of injury in the ischemic liver. Because **caspases**, calpains, and Bcl-2 have a pivotal role in the regulation of apoptosis, we hypothesized that ischemic preconditioning protects by inhibition of apoptosis through down-regulation of **caspase** and calpain activities and up-regulation of Bcl-2. A preconditioning period of 10 minutes of ischemia followed by 15 minutes of reperfusion maximally protected livers subjected to prolonged ischemia. After reperfusion, serum aspartate transaminase (AST) levels were reduced up to 3-fold in preconditioned animals. All animals subjected to 75 minutes of ischemia died, whereas all those who received ischemic preconditioning survived. Apoptosis of hepatocytes and sinusoidal endothelial cells, assessed b y in situ TUNEL assay and DNA fragmentation by gel electrophoresis, was dramatically reduced with preconditioning. **Caspase** activity, measured by poly (adenosine diphosphate ribose) polymerase (PARP) proteolysis and a specific **caspase**-3 **fluorometric assay**, was inhibited by ischemic preconditioning. The antiapoptotic mechanism did not involve calpain-like activity or Bcl-2 expression because levels were similar in control and preconditioned livers. In conclusion, ischemic preconditioning confers dramatic protection against prolonged ischemia via inhibition of apoptosis through down-regulation of **caspase** 3 activity, independent of calpain-like activity or Bcl-2 expression.

L4 ANSWER 37 OF 45 MEDLINE on STN DUPLICATE 22
1999238883 Document Number: 99238883. PubMed ID: 10220501.
Gastrointestinal safety of nitric oxide-derived aspirin is related to inhibition of ICE-like cysteine proteases in rats. Fiorucci S; Antonelli E; Santucci L; Morelli O; Miglietti M; Federici B; Mannucci R; Del Soldato P; Morelli A. (Sezione di Gastroenterologia ed Epatologia, Dipartimento di Medicina Clinica e Sperimentale, Universita degli Studi di Perugia, Perugia, Italy.. Gastrol@unipg.it) . GASTROENTEROLOGY, (1999 May) 116 (5) 1089-106. Journal code: 0374630. ISSN: 0016-5085. Pub. country: United States. Language: English.

AB BACKGROUND & AIMS: **Caspases**, a class of cysteine proteases, modulate apoptosis. Nitric oxide (NO)-releasing nonsteroidal anti-inflammatory drugs (NSAIDs) are a new class of NSAID derivatives with reduced gastrointestinal toxicity. The aim of this study was to investigate whether cysteine endoproteases are involved in the pathogenesis of NSAID gastropathy and are target for NO-aspirin (NCX-4016). METHODS: Rats were treated orally with aspirin or equimolar doses of NCX-4016. **Caspase** activities were measured by **fluorometric assay**. Apoptosis was quantified by an enzyme-linked immunosorbent assay for histone-associated DNA, DNA ladder on agarose gel, and terminal deoxynucleotidyl transferase-mediated

deoxyuridine triphosphate nick-end labeling assay. A primary culture of gastric chief cells was used to investigate whether NCX-4016 modulates guanosine 3',5'-cyclic monophosphate (cGMP)-dependent pathways. RESULTS: Short- and long-term (7 days) aspirin administration resulted in a time- and dose-dependent gastric injury that was associated with apoptosis and **caspase** up-regulation. Z-VAD.FMK, a pancaspase inhibitor, and NO donors protected from acute damage induced by aspirin. NCX-4016 spared the gastric mucosa and caused **caspase** inactivation by S-nitrosylation. Inhibition of tumor necrosis factor (TNF)-alpha release or activity by TAPI-2 or anti-TNF-alpha receptor monoclonal antibodies protected against mucosal damage and **caspase** activation. NCX-4016 protected gastric chief cells from toxicity induced by TNF-alpha by activating cGMP-dependent pathways. CONCLUSIONS: Aspirin administration leads to a TNF-alpha-dependent activation of gastric **caspases**. NO-aspirin spares the gastric mucosa and inhibits **caspase** activity through cGMP-dependent and -independent pathways.

L4 ANSWER 38 OF 45 MEDLINE on STN DUPLICATE 23

1999441481 Document Number: 99441481. PubMed ID: 10511810. Evaluation of cytarabine-induced apoptosis in leukemic cell lines; utility of annexin V method. Akiyama H; Suzuki K; Ino T; Katsuda I; Hirano M. (Department of Medicine, Fujita Health University School of Medicine/School of Hygiene.) RINSHO BYORI. JAPANESE JOURNAL OF CLINICAL PATHOLOGY, (1999 Aug) 47 (8) 774-9. Journal code: 2984781R. ISSN: 0047-1860. Pub. country: Japan. Language: Japanese.

AB Apoptosis is a morphologically and biochemically distinct form of cell death that occurs under a variety of physiological and pathological conditions. In the present study, using leukemic cell lines, time course of cytarabine-induced apoptosis was examined morphologically, using annexin V method, TUNEL method and **fluorometric assay** for **caspase**-3 activity. Morphological changes characteristic of apoptosis were observed in U937 and HL60 cells after 4-hour incubation with cytarabine and progressively evident until 48-hour incubation, but rarely found in K562 cells. In annexin V method and assay for **caspase**-3 activity, changes accompanied by apoptosis could also be detected at 4-hour incubation with cytarabine, but in TUNEL method, they were not found until 24-hour incubation. The advantage of annexin V method which detects phosphatidylserine emerging on cell surface during the early course of apoptosis included simplicity and rapidity of the procedure and short time requirement for apoptosis to appear after incubation with cytarabine. Usefulness of annexin V method in a study of clinical samples was discussed.

L4 ANSWER 39 OF 45 MEDLINE on STN DUPLICATE 24

1999311015 Document Number: 99311015. PubMed ID: 10381624. Purification and catalytic properties of human **caspase** family members. Garcia-Calvo M; Peterson E P; Rasper D M; Vaillancourt J P; Zamboni R; Nicholson D W; Thornberry N A. (Department of Enzymology, Merck Research Laboratories, R80W-250, P.O. Box 2000, Rahway, New Jersey 07065, USA.. margarita_garcia-calvo@merck.com) . CELL DEATH AND DIFFERENTIATION, (1999 Apr) 6 (4) 362-9. Journal code: 9437445. ISSN: 1350-9047. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Members of the **caspase** family of cysteine proteases are known to be key mediators of mammalian inflammation and apoptosis. To better understand the catalytic properties of these enzymes, and to facilitate the identification of selective inhibitors, we have systematically purified and biochemically characterized ten homologues of human origin (**caspases** 1 - 10). The method used for production of most of these enzymes involves folding of active enzymes from their constituent subunits which are expressed separately in E. coli, followed by ion exchange chromatography. In cases where it was not possible to use this method (**caspase**-6 and -10), the enzymes were instead expressed as soluble

proteins in E. coli, and partially purified by ion exchange chromatography. Based on the optimal tetrapeptide recognition motif for each enzyme, substrates with the general structure Ac-XEXD-AMC were used to develop continuous **fluorometric assays**. In some cases, enzymes with virtually identical tetrapeptide specificities have kcat/Km values for fluorogenic substrates that differ by more than 1000-fold. Using these assays, we have investigated the effects of a variety of environmental factors (e.g. pH, NaCl, Ca2+) on the activities of these enzymes. Some of these variables have a profound effect on the rate of catalysis, a finding that may have important biological implications.

L4 ANSWER 40 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1999:231830 Document No.: PREV199900231830. **Caspases**: Preparation and characterization. Stennicke, Henning R.; Salvesen, Guy S. [Reprint author]. Program for Apoptosis and Cell Death Research, Burnham Institute, 10901 North Torrey Pines Road, La Jolla, CA, 92037, USA. Methods (Orlando), (April, 1999) Vol. 17, No. 4, pp. 313-319. print.
CODEN: MTHDE9. ISSN: 1046-2023. Language: English.
AB **Caspases** and their involvement in programmed cell death have been an area of significant interest since their initial identification in 1992. To facilitate the search for new components involved in cell death, and to aid researchers in understanding the interactions between currently known cell death proteins, we describe a number of techniques commonly used in the preparation and characterization of **caspases**.

L4 ANSWER 41 OF 45 MEDLINE on STN DUPLICATE 25
1999330354. PubMed ID: 10403560. Correlation of tumor necrosis factor alpha (TNF alpha) with high **Caspase** 3-like activity in myelodysplastic syndromes. Mundle S D; Reza S; Ali A; Mativi Y; Shetty V; Venugopal P; Gregory S A; Raza A. (Rush Cancer Institute, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612, USA.. smundle@rush.edu) . Cancer letters, (1999 Jun 1) 140 (1-2) 201-7. Journal code: 7600053. ISSN: 0304-3835. Pub. country: Ireland. Language: English.
AB Increased intramedullary apoptotic death of hematopoietic cells is thought to contribute to the ineffective hematopoiesis in myelodysplastic syndromes (MDS). Furthermore, high amounts of tumor necrosis factor alpha (TNF alpha) have previously been correlated with apoptosis in MDS marrows. The present studies were undertaken to examine the status of two key downstream effectors of TNF alpha signaling, i.e. **Caspase** 1 and **Caspase** 3 enzymes, using a **fluorometric assay** in the bone marrow aspirate mononuclear cells (BMMNC) in relation to apoptotic DNA fragmentation detected by in situ end-labeling (ISEL) of DNA and with localization of TNF alpha in the corresponding biopsies from 14 MDS patients. Both **Caspase** 1 and 3 were detectable in freshly harvested BMMNC, albeit median **Caspase** 3 levels (47.5 units/mg protein) being almost 10 times higher than **Caspase** 1 (4.0 units/mg protein). Upon short-term culture for 4 h in a serum-supplemented medium in vitro a significant increase was seen in **Caspase** 3 activity (58.8 +/- 13.9 at 0 h vs. 177.8 +/- 55.2 units/mg protein at 4 h, n = 14, P = 0.017) and in percent cells labeled by ISEL (apoptotic index or AI%: 0.76% +/- 0.25% vs. 3.99% +/- 1.1%, n = 14, P = 0.004, respectively). **Caspase** 1 activity increased after 15 min in culture. Interestingly, TNF alpha levels measured by immunohistochemistry correlated with the net increase in **Caspase** 3 activity after 4 h (p = 0.517, n = 13, P = 0.07) and the starting levels of **Caspase** 1 at 0 h correlated with the **Caspase** 3 levels attained at 4 h (p = 0.593, n = 13, P = 0.033). Additionally when TNF alpha-positive bone marrows (8/14) were compared with the negative marrows (6/14) the **Caspase** 3 levels were significantly higher in the TNF alpha-positive marrows (189.6 +/- 66.2 vs. 25.0 +/- 14.6 units/mg protein, respectively, P = 0.043). The increase in AI%, though not

statistically significant, was also higher in the TNF alpha-positive marrows. Finally in HL60 cells the effects of different **Caspase** inhibitors and pentoxifylline (PTX) (interferes with lipid signaling of cytokines) on TNF alpha-induced apoptosis were evaluated. TNF alpha treatment significantly increased AI% (P < 0.003) as compared to the untreated controls. A co-treatment with three **Caspase** inhibitors, zVAD.FMK (inhibitor of **Caspases** 1 and 3, 10 microM/l), Ac.YVAD.FMK (**Caspase** 1 inhibitor, 1 microM/l), Ac.DEVD.FMK (**Caspase** 3 inhibitor, 10 microM/l) as well as PTX (250 microM/l) significantly curtailed the AI% induced by TNF alpha. The present studies thus identify the downstream effectors of TNF alpha-inducible apoptosis in MDS and so also the suppressors of TNF alpha apoptotic signaling. These results may have significant clinical implications in the therapy of MDS in the future.

L4 ANSWER 42 OF 45 MEDLINE on STN DUPLICATE 26

1999239158. PubMed ID: 10222652. The relative extent and propensity of CD34+ vs. CD34- cells to undergo apoptosis in myelodysplastic marrows. Mundle S; Venugopal P; Shetty V; Ali A; Chopra H; Handa H; Rose S; Mativi B Y; Gregory S A; Preisler H D; Raza A. (Rush Cancer Institute, Rush Presbyterian-St. Luke's Medical Center, Chicago, Illinois 60612, USA.. smundle@rpslmc.edu) . International journal of hematology, (1999 Apr) 69 (3) 152-9. Journal code: 9111627. ISSN: 0925-5710. Pub. country: Ireland. Language: English.

AB The paradox of peripheral cytopenias despite cellular bone marrow (BM) observed in myelodysplastic syndromes (MDS) has been associated with excessive intramedullary apoptosis of hematopoietic cells. Since MDS is regarded as a stem cell disorder, the present studies were undertaken to examine the relative susceptibility and propensity of early progenitor CD34+ cells to undergo apoptosis as compared to more maturing/matured CD34- cells. Five serial studies were performed on 4 independent groups of 36 newly diagnosed MDS patients. First, in 2 separate groups of 16 and 8 patients each, measurement of the extent of apoptosis in CD34+ and CD34- fractions of the BM aspirate mononuclear cells was carried out using independent biparametric flow cytometry methods, CD34 labeling/terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) (n = 16), and CD34 labeling/reduced uptake of nucleic acid staining dye LDS751 (n = 8). The difference in the median degrees of apoptosis in CD34+ vs. CD34- cells was not statistically significant by either technique (P = 0.583 and P = 0.674 for TUNEL and LDS751, respectively). In the next group of 4 MDS patients, a double-labeling was performed on plastic embedded marrow biopsy sections, to detect CD34 antigen with specific monoclonal antibody and apoptosis by in situ end labeling (ISEL) of fragmented DNA. Despite high overall apoptosis (56.2% +/- 18.4%), only an occasional CD34+ cell was found to be simultaneously labeled with ISEL. Finally, in the last group of 8 MDS patients, CD34+ cells were separated from CD34- cells on affinity column and cultured in serum containing medium for 4 hours. At 0- and 4-hour time points, ISEL was carried out to label apoptotic cells. In addition, a **fluorometric assay** was employed to estimate the activity of a proapoptotic enzyme, **Caspase** 3. Both the net increase in % ISEL labeled cells (apoptotic index or AI) and **Caspase**-3 activity were significantly lower in CD34+ cells as compared to CD34- cells (AI, 0.87% +/- 0.5% vs. 3.97% +/- 1.4%, n = 6, P = 0.028 and **Caspase**-3 Units/mg protein, 46.9 +/- 25.0 vs. 71.7 +/- 23.03, n = 5, P = 0.042, respectively). We conclude that when estimated in a total population of mononuclear cells, CD34+ cells and CD34- cells show comparable degrees of apoptosis. However, once separated the CD34+ fraction demonstrates lower propensity to undergo apoptosis, thereby suggesting the CD34- fraction as being a possible source for proapoptotic signaling.

L4 ANSWER 43 OF 45 MEDLINE on STN DUPLICATE 27

1999336751 Document Number: 99336751. PubMed ID: 10410970. Application of a **fluorometric assay** to detect **caspase** activity in thymus tissue undergoing apoptosis in vivo. Gorman A M; Hirt U A; Zhivotovsky B; Orrenius S; Ceccatelli S. (Institute of Environmental Medicine, Division of Toxicology, Karolinska Institutet, Stockholm, Sweden.) JOURNAL OF IMMUNOLOGICAL METHODS, (1999 Jun 24) 226 (1-2) 43-8. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB To date, in vivo apoptosis within the thymus has been assessed using morphological criteria and/or detection of a DNA ladder indicative of oligonucleosomal fragmentation of the DNA. Here, we have used a fluorometric method to investigate activation of the **caspase** protease family in the thymus following in vivo induction of apoptosis by injection of the synthetic glucocorticoid hydrocortisone. Cleavage of DEVD-MCA by **caspase**-3 and other group II **caspases** releases free MCA which can be detected fluorimetrically. We demonstrate a time-dependent increase in DEVD-MCA cleavage activity within this tissue indicating the activation of **caspase**-3 like enzymes. This activity was inhibited by the specific group II **caspase** inhibitor DEVD-CHO. The interpretation of increased **caspase** activity was confirmed by immunoblot analysis to reveal cleavage of the **caspase**-3 substrate, fodrin. In addition, agarose gel electrophoresis of the DNA yielded a ladder pattern, confirming the occurrence of apoptosis. This study demonstrates that DEVD-MCA cleavage activity may be a useful quantitative method for the analysis of apoptosis in thymus tissue. It is a relatively rapid procedure not requiring thymocyte isolation or gel electrophoresis and detects fairly early biochemical changes occurring during apoptosis. In the present study we have used this method to demonstrate the involvement of **caspases** in thymocyte apoptotic death induced in vivo by glucocorticoids. Thus, measurement of **caspase** activity in thymus tissue may have applications for studying the in vivo effects of immunotoxicants.

L4 ANSWER 44 OF 45 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1999:42992 Document No.: PREV199900042992. Molecular cloning and identification of murine **caspase**-8. Van De Craen, Marc; Van Loo, Geert; Declercq, Wim; Schotte, Peter; Van Den Brande, Ilse; Mandruzzato, Susanna; Van Der Bruggen, Pierre; Fiers, Walter; Vandenabeele, Peter [Reprint author]. Dep. Mol. Biol., Interuniversity Inst. Biol., Univ. Ghent, K. L. Ledeganckstraat 35, B-9000 Ghent, Belgium. Journal of Molecular Biology, (Dec. 11, 1998) Vol. 284, No. 4, pp. 1017-1026. print. CODEN: JMOBAK. ISSN: 0022-2836. Language: English.

AB Several **caspases** are mediators of apoptotic cell death. We describe a novel murine member of this growing protein family. Based on homology and especially on the substrate specificity, this new procaspase is identified as the murine counterpart of human procaspase-8. The protein exhibits a rather low similarity (76%) and identity (70%) to human procaspase-8. Procaspase-8 mRNA is expressed in all adult mouse tissues examined, the highest levels being reached in kidney, liver and lung. Procaspase-8 mRNA expression is highest in seven-day old embryos, but also during later stages of development the expression was fairly high. Both human and murine procaspase-8 are very weak substrates for granzyme B as compared to procaspase-3. Murine procaspases-1, 2, 3, 6, 7, 8, 11/4 and 12 are processed by recombinant murine **caspase**-8, suggesting a key role in the procaspase activation cascade. In addition, murine **caspase**-8 induced cell death that was inhibited both by cytokine response modifier A and p35. In vitro experiments demonstrated that p35 inhibits **caspase**-8 directly.

L4 ANSWER 45 OF 45 MEDLINE on STN DUPLICATE 28
97383261. PubMed ID: 9236222. The role of CED-3-related cysteine proteases in apoptosis of cerebellar granule cells. Eldadah B A; Yakovlev A G; Faden

A I. (Georgetown Institute for Cognitive and Computational Sciences, Georgetown University Medical Center, Washington, DC 20007, USA.) Journal of neuroscience : official journal of the Society for Neuroscience, (1997 Aug 15) 17 (16) 6105-13. Journal code: 8102140. ISSN: 0270-6474. Pub. country: United States. Language: English.

AB The CED-3-related cysteine proteases (CRCPs) have been implicated as mediators of apoptosis, primarily in hematogenous cell systems, but their role in neuronal apoptosis remains unclear. The present study examined the role of two CRCP families-CPP32- and interleukin-1beta converting enzyme (ICE)-like cysteine proteases-in apoptosis of cerebellar granule cells (CGCs) caused by withdrawal of serum and/or potassium (K+). Serum deprivation potentiated apoptosis caused by K+ withdrawal, reducing cell viability by approximately one half of control values after 12 hr as measured by calcein fluorescence. Cell death after serum/K+ deprivation was significantly attenuated by the CPP32-like inhibitor z-DEVD-fmk; however, the ICE-like inhibitor z-YVAD-fmk had only slightly protective effects at the highest concentration used. Both inhibitors reduced CPP32-like activity directly in an in vitro **fluorometric assay** system, although z-DEVD-fmk showed much greater potency. K+ and serum/K+ deprivation each were accompanied by increased CPP32-like activity; however, ICE-like activity was absent after 12 hr of serum and/or K+ deprivation. CPP32 mRNA levels were unchanged after K+ deprivation but increased after serum and combined serum/K+ withdrawal as measured by reverse transcription-PCR (RT-PCR), with peak values at 4 hr reaching 210 +/- 37% and 269 +/- 42% of control levels, respectively. In contrast, ICE mRNA was undetectable by RT-PCR. These results are consistent with the hypothesis that CPP32-like proteases play an important role in apoptosis of CGCs caused by deprivation of K+ or serum/K+.

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=> s l1 and drug screening
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L5          36 L1 AND DRUG SCREENING

=> dup remomove l5
ENTER REMOVE, IDENTIFY, ONLY, OR (?):l5
'L25' IS NOT VALID HERE
Enter "REMOVE" to identify and remove duplicate answers.
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Enter "ONLY" to identify and create an answer set containing only
duplicate records.
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'REMOMOVE' IS NOT VALID.  VALID FILE NAMES ARE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH,
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You have entered a file name of duplicates to keep that is not
referenced by any of the L#s specified for this DUPLICATE command.
The file names of duplicates that can be kept are listed above.
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L6           25 DUP REMOVE L5 (11 DUPLICATES REMOVED)

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L6   ANSWER 1 OF 25  EMBASE  COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
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on STN
2003001860 EMBASE A novel high throughput screening assay for HCV NS3 serine protease inhibitors. Berdichevsky Y.; Zemel R.; Bachmatov L.; Abramovich A.; Koren R.; Sathiyamoorthy P.; Golan-Goldhirsh A.; Tur-Kaspa R.; Benhar I.. R. Tur-Kaspa, Molecular Hepatology Res. Laboratory, Sackler School of Medicine, Tel-Aviv University, Ramat Aviv 69978, Israel. benhar@post.tau.ac.il. Journal of Virological Methods 107/2 (245-255) 2003.
Refs: 48.
ISSN: 0166-0934. CODEN: JVMEDH.
Publisher Ident.: S 0166-0934(02)00255-0. Pub. Country: Netherlands. Language: English. Summary Language: English.

AB Hepatitis C virus (HCV) infection is a major worldwide health problem, causing chronic hepatitis, liver cirrhosis and primary liver cancer (Hepatocellular carcinoma). HCV encodes a precursor polyprotein that is enzymatically cleaved to release the individual viral proteins. The viral non-structural proteins are cleaved by the HCV NS3 serine protease. NS3 is regarded currently as a potential target for anti-viral drugs thus specific inhibitors of its enzymatic activity should be of importance. A prime requisite for detailed biochemical studies of the protease and its potential inhibitors is the availability of a rapid reliable in vitro assay of enzyme activity. A novel assay for measurement of HCV NS3 serine protease activity was developed for screening of HCV NS3 serine protease potential inhibitors. Recombinant NS3 serine protease was isolated and purified, and a **fluorometric assay** for NS3 proteolytic activity was developed. As an NS3 substrate we engineered a recombinant fusion protein where a green fluorescent protein is linked to a cellulose-binding domain via the NS5A/B site that is cleavable by NS3. Cleavage of this substrate by NS3 results in emission of fluorescent light that is easily detected and quantitated by fluorometry. Using our system we identified NS3 serine protease inhibitors from extracts obtained from natural Indian Siddha medicinal plants. Our unique **fluorometric assay** is very sensitive and has a high throughput capacity making it suitable for screening of potential NS3 serine protease inhibitors.

L6 ANSWER 2 OF 25 EMBASE
on STN
2003210597 EMBASE Determining acetylcholinesterase inhibitory activity in Plant extracts using a fluorimetric flow assay. Rhee I.K.; Appels N.; Luijendijk T.; Irth H.; Verpoorte R.. R. Verpoorte, Division of Pharmacognosy, Leiden/Amsterdam Ctr. for Drug Res., Leiden University, PO Box 9502, 2300 RA Leiden, Netherlands. verpoort@LACDR.leidenuniv.nl. Phytochemical Analysis 14/3 (145-149) 2003.
Refs: 18.
ISSN: 0958-0344. CODEN: PHANEL. Pub. Country: United Kingdom. Language: English. Summary Language: English.

AB A **fluorometric assay** for acetylcholinesterase inhibitory activity was developed in a flow system using the fluorogenic substrate 7-acetoxy-1-methyl quinolinium iodide which is hydrolysed to the highly fluorescent 7-hydroxy-1-methyl quinolinium iodide. The detection limit of galanthamine is 0.5 μM, which is about 20 times more sensitive than in the colorimetric flow assay. In the presence of 30% methanol or of 5% acetonitrile, about 70% of the enzyme activity could still be detected. Various plant extracts have been screened using the described system including bulbs of Galanthus nivalis, Eucharis amazonica (E. x grandiflora), Crinum powelli and Nerine bowdenii (all members of the Amaryllidaceae), which showed strong AchE inhibitory activity. Copyright

L6 ANSWER 3 OF 25 BIOSIS on STN
2003:264210 Document No.: PREV200300264210. Applications of recombinant

Leishmania amazonensis expressing egfp or the beta-galactosidase gene for **drug screening** and histopathological analysis. Okuno, Takahiro; Goto, Yasuyuki; Matsumoto, Yoshitsugu; Otsuka, Haruki; Matsumoto, Yasunobu [Reprint Author]. Laboratory of Global Animal Resource Science, Graduate School of Agricultural and Life Sciences, University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo, 113-8657, Japan. Experimental Animals (Tokyo), (April 2003) Vol. 52, No. 2, pp. 109-118. print. ISSN: 1341-1357 (ISSN print). Language: English.

AB Leishmania amazonensis recombinants expressing the enhanced green fluorescent protein (egfp) gene or beta-galactosidase gene (lacZ) were constructed for **drug screening** and histopathological analysis. The egfp or lacZ in a leishmanial transfection vector, p6.5, was introduced into L. amazonensis promastigotes, and egfp or lacZ-carrying recombinant L. amazonensis, La/egfp and La/lacZ, respectively, were obtained. Expression of egfp or lacZ in both promastigotes and amastigotes could be clearly visualized by fluorescence microscopy or by light microscopy with 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside (X-Gal), respectively. Fluorescence signal and beta-galactosidase activity measured by a colorimetric reaction with chlorophenol red beta-D-galactopyranoside (CPRG) were well correlated to the numbers of these parasites. The inhibitory concentration (IC50) of a leishmanicidal drug, amphotericin B, in L. amazonensis promastigotes measured using La/egfp and La/lacZ was similar to that measured by conventional methods such as cell counting, thymidine incorporation and colorimetric assay. Furthermore, the fluorescence signal and absorbance of CPRG correlated well with the numbers of La/egfp and La/lacZ amastigotes in macrophages, respectively, suggesting La/egfp and La/lacZ can be a convenient and useful tool for **drug screening** not only in promastigotes, but also in amastigotes of L. amazonensis. La/lacZ collected from mouse tissues four weeks after the parasite infection were stained well with X-Gal. La/lacZ allowed parasite detection at high sensitivity in the tissues of infected mice and will be useful for following infections in macrophages in vivo. Thus, the marker-transfected Leishmania parasites constructed in this study will be useful for analyses of Leishmania parasites, especially at the intracellular stage.

L6 ANSWER 4 OF 25 MEDLINE on STN

2003084881 Document Number: 22463828. PubMed ID: 12576927. Radiation enhancement by gemcitabine-mediated cell cycle modulations. Mose Stephan; Class Reiner; Weber Hans-Walter; Rahn Angelika; Brady Luther W; Bottcher Heinz D. (Department of Radiation Oncology, Johann Wolfgang Geothe-University, Grankfurt/Main, Germany.) AMERICAN JOURNAL OF CLINICAL ONCOLOGY, (2003 Feb) 26 (1) 60-9. Journal code: 8207754. ISSN: 0277-3732. Pub. country: United States. Language: English.

AB The purpose of this study was to investigate the exact dose dependency and time dependency of the radiation-enhancing effect of gemcitabine (2',2'difluoro desoxycytidine [dFdC]) in in vitro experiments (HeLa cells: cancer of the uterine cervix, #4197 cells: oropharyngeal squamous cell carcinoma), and to correlate this effect with the underlying changes in cell cycle distribution. Cell viability was determined fluorometrically after exposure to dFdC (0-20.0 micro mol/l), irradiation (0-37.5 Gy), and both modalities. Combining both therapies, cells were exposed to dFdC (0-10.0 micro mol/l) for 24 hours before further treatment and irradiated (0-30 Gy) immediately afterwards with or without removal of dFdC. For cell cycle analysis by flow cytometry, cells were irradiated (0-40 Gy) or treated with dFdC (0.012-1.0 micro mol/l, 24-48 hours). Additionally, cells were exposed to dFdC (2.0 micro mol/l, 0-4 hours). Cell cycle kinetics were evaluated using bromodeoxyuridine (BrdU) (10 micro mol/l) S-phase labeling, given either 30 minutes before or in the last hour of dFdC treatment (2.0 micro mol/l, 0-6 hours). The **fluorometric assay** revealed that dFdC enhances radiation-induced cytotoxicity

at marginally toxic or nontoxic concentrations (<37 nmol/l). Radiation resulted in the anticipated G2/M arrest already at 2 Gy. DFdC induced concentration and exposure time-dependent cell cycle changes that were better resolved using BrdU, demonstrating a pronounced S-phase arrest already at 12 nmol/l. BrdU-pulse labeling revealed that the cell cycle block occurred at the G1/S boundary. Our data reconfirm the already known radiation enhancement, the S-phase specific activities of dFdC, and the relevance of the synchronized progression of cells through the S-phase with regard to the radiosensitizing properties of low-dose dFdC. However, we could demonstrate that before progressing in the S-phase, cells were blocked and partially synchronized at the more radiosensitive G1/S boundary. Furthermore, cells progressing past the block might accumulate proapoptotic signals caused by both radiation and dFdC, which will also results in cell death.

L6 ANSWER 5 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN
2002:123251 Document No. 136:163288 A **fluorometric assay** for cytochrome P 450 2C19 and cytochrome P 450 2C9 using 3-butyryl-7-methoxycoumarin and application to high throughput inhibition screening assays. Bloomer, Jacqueline Carol; Leach, Colin Andrew (Smithkline Beecham PLC, UK). PCT Int. Appl. WO 2002012542 A2 20020214, 10 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-EP8788 20010730. PRIORITY: GB 2000-19475 20000808.

AB A coumarin derivative, 3-butyryl-7-methoxycoumarin (I), has now been identified which is an improved substrate for cytochrome P 450 isoenzymes CYP2C19 and CYP2C9 and which is of use for configuring high throughput inhibition screening assays. O-dealkylation of I following incubation with CYP2C19 and CYP2C9 gives a readily quantifiable fluorescent product, 3-butyryl-7-hydroxycoumarin.

L6 ANSWER 6 OF 25 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 1
2002166122 EMBASE Effects of intrinsic fluorescence and quenching on fluorescence-based screening of natural products. Zou L.; Harkey M.R.; Henderson G.L.. Dr. G.L. Henderson, Department of Medical Pharmacology, School of Medicine, University of California, Davis, CA 95616, United States. glhenderson@ucdavis.edu. Phytomedicine 9/3 (263-267) 2002. Refs: 7.
ISSN: 0944-7113. CODEN: PYTOEY. Pub. Country: Germany. Language: English. Summary Language: English.

AB To evaluate the effects of intrinsic (natural) fluorescence and quenching as confounding variables in fluorescence-based enzyme inhibition assays of natural products, we measured the fluorescence and quenching properties of 25 components of popular herbal products. The analyses were performed under conditions typically employed in drug-drug interaction studies that use c-DNA-derived P450 isoforms and surrogate fluorogenic substrates. Four of the 25 compounds tested (isorhamnetin, quercetin, vitexin, and yangonin) fluoresced or quenched sufficiently to interfere with these assays. Intrinsic fluorescence had a greater effect on these assays than quenching and for one compound, yangonin, was sufficient to mask inhibition and potentially produce a false negative result. Quenching had less of an effect on these assays, but was significant enough for one compound, quercetin, to mimic "weak" inhibition. Therefore, because intrinsic fluorescence or quenching could render some natural products unsuitable for testing in certain **fluorometric assays**,

it would be prudent to include an evaluation of these properties in experimental protocols.

L6 ANSWER 7 OF 25 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 2
2002:389835 Document No.: PREV200200389835. A new automatized **fluorometric assay** for anti-Leishmania **drug screening**. Le Pape, Patrice [Reprint author]; Pagniez, Fabrice; Abdala, Hiam. Laboratoire de Parasitologie et Organisation Animale, Faculte de Pharmacie, UPRES EA 1155, 1 Rue Gaston Veil, 44035, Nantes Cedex, France. plepape@sante.univ-nantes.fr. Acta Parasitologica, (January, 2002) Vol. 47, No. 1, pp. 79-81. print.
ISSN: 1230-2821. Language: English.
AB The therapeutic armentarium against leishmaniasis is very restricted, using mainly up to now drugs showing several side effects and resistance. Consequently, there is a real need to find new compounds as alternatives for the treatment of leishmaniasis. However, most of the classic antileishmanial primary screening assays are not suitable for measuring the cytotoxicity of large number of drug candidates because of the manipulations required. We have established a new assay that incorporate a fluorometric growth indicator based on the detection of a metabolic activity in culture medium after the chemical reduction of alamar Blue(R) by cells. This antileishmanial test was evaluated on amphotericin B, meglumine antimoniate, allopurinol, ketoconazole and edelfosine. The results reported show the advantages of this fluorochrome on the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) for Leishmania cytotoxicity measurement. Moreover, this study is the first report of the use of alamar Blue(R) fluorogenic assay for activity assessment of potential antileishmanial drugs against promastigotes.

L6 ANSWER 8 OF 25 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
2001228261 EMBASE Anti-tumor activity of defensin on gastric cancer cell line in vitro. Wen Chao Liu; Huai Xing Mu; Ren J.; Xue Yong Zhang; Bo Rong Pan. W.C. Liu, Department of Oncology, Xijing Hospital, Fourth Military Medical University, Xian 710033, China. liuch@fmmu.edu.cn. World Chinese Journal of Digestology 9/6 (622-626) 2001.
Refs: 50.
ISSN: 1009-3079. CODEN: SHXZF2. Pub. Country: China. Language: Chinese. Summary Language: English; Chinese.
AB AIM: To testify and observe the cytolytic activity of small peptide molecule termed as defensins generated from polymorphonuclear neutrophils (PMN) on gastric cancer cell line and/ or the resistant cell line. METHODS: The peripheral blood was collected from healthy blood donors. The PMNs were then homogenized and extracted by centrifugation. The defensin was finally prepared from liquid chromatography. The tetrazolium blue colorimetric assay (MTT) was introduced to evaluate the cytotoxicity of defensin on gastric cancer cell line SGC-7901 and the resistant partner cell line SGC-7901/VCR. The flow cytometry was employed to analyse the cell cycle. The oxygen and reactive nitrogen relevant with lipid peroxidative MDA and NO derived from the cultured gastric cancer cell line exposure to defensin treatment was performed via **fluorometric assay**. RESULTS: The purified defensins of M(r) 3500 had good antimicrobiol activity. The anti-tumor experiment of defensins showed that defensins exerted a strong antitumor activity on the cell lines in a concentration and time dependent manner. Flow cytometrical analysis revealed that the proportion of S-phase was decreased among the cells treated. The production of MDA and NO was much higher than that of control group and a lineal dose-dependent correlation was seen. CONCLUSION: Our findings laid a new ground that the defensin has confirmed antitumor activity except for these known antimicrobial effect. The data available

in this report might extend the conceptual recognization when defensin is concerned. The bio-function of the defensin on the tumors still needs to be explored further.

L6 ANSWER 9 OF 25 MEDLINE on STN
2001407845 Document Number: 21351821. PubMed ID: 11459200. Arsenic compounds induce cytotoxicity and apoptosis in cisplatin-sensitive and -resistant gynecological cancer cell lines. Du Y H; Ho P C. (Department of Pharmacy, National University of Singapore, Singapore.) CANCER CHEMOTHERAPY AND PHARMACOLOGY, (2001 Jun) 47 (6) 481-90. Journal code: 7806519. ISSN: 0344-5704. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB PURPOSE: Arsenic compounds have been found to be effective in the treatment of acute promyelocytic leukemia through the downregulation of bcl-2 expression. Resistant ovarian cancer cells often overexpress bcl-2 or p53 proteins or both. We hypothesized that arsenic compounds, such as As2O3 and As2S3, could also be active against gynecological cancers resistant to conventional chemotherapy. METHODS: We investigated the effects of these two arsenic compounds in vitro on ovarian cancer cell lines sensitive (OVCAR, GG, JAM) and resistant (CI80-13S) to cisplatin (CDDP) and on human cervical cancer cell lines (HeLa) in comparison with their effects on human fibroblasts (HF). A **fluorometric assay** based on measurements of fluorescein diacetate (FDA) in cells was used to determine cell viability. Apoptosis was assessed in terms of cell morphology, by flow cytometry and by a DNA fragmentation assay. RESULTS: Treatment of each cell line with the As2O3 or As2S3 led to a marked dose-dependent decrease in cell growth. The IC50 of the two compounds indicated a significantly greater cytotoxic effect against all the cancer cells tested than against the normal HF. At a clinically achievable concentration (2 microM), As2O3 selectively inhibited the growth and induced apoptosis in CI80-13S, OVCAR and HeLa cells but had no significant apoptotic effect on GG or JAM cells or HF. Following treatment with 5 microM As2S3, the CI80-13S, OVCAR and HeLa cells also exhibited growth inhibition and induction of apoptosis. CONCLUSIONS: Arsenic compounds (As2O3 and As2S3) can inhibit growth and induce apoptosis in human ovarian and cervical cancer cells at clinically achievable concentrations, indicating that As2O3 and As2S3 could be effective in the treatment of gynecological cancer.

L6 ANSWER 10 OF 25 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2001:976015 The Genuine Article (R) Number: 498FX. Detection of a decrease in green fluorescent protein fluorescence for the monitoring of cell death: An assay amenable to high-throughput screening technologies. Steff A M; Fortin M; Arguin C; Hugo P (Reprint). PROCREA BioSci, Div Res & Dev, 6100 Royalmt, Montreal, PQ H4P 2R2, Canada (Reprint); PROCREA BioSci, Div Res & Dev, Montreal, PQ H4P 2R2, Canada. CYTOMETRY (1 DEC 2001) Vol. 45, No. 4, pp. 237-243. Publisher: WILEY-LISS. DIV JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012 USA. ISSN: 0196-4763. Pub. country: Canada. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background: Reliable assessment of cell death is now pivotal to many, research programs aiming at generating new anti-tumor compounds or at screening cDNA libraries. Stich approaches need to rely on reproducible, easy-to-handle, and rapid microplate-based cytotoxicity assays that are an-tenable to high-throughput screening (HTS) technologies. We describe it method for the direct measurement of cell death, based on the detection of a decrease in fluorescence observed following death induction in cells expressing enhanced green fluorescent protein (EGFP).

Methods: Cell death was induced by it variety of apoptotic stimuli in various EGFP-expressing mammalian cell lines, including those routinely used in anti-cancer **drug screening**. Decrease in fluorescence was assessed either by flow cytometry (and compared with

other apoptotic markers) or by a fluorescence microplate reader.
Results: Cells expressing EGFP exhibited a decrease in fluorescence when treated by various agents, such as chemotherapeutic drugs, UV irradiation, or caspase-independent cell death inducers. Kinetics and sensitivity of this EGFP-based assay were comparable to those of traditional apoptosis markers such as annexin-V binding, pro. gen species pidium iodide incorporation, or reactive oxygen production. We also show that the decrease in EGFP fluorescence is directly quantifiable in a fluorescence-based microplate assay. Furthermore, analysis of EGFP protein content in cells undergoing cell death demonstrates that the decrease in fluorescence does not arise from degradation of the protein.
Conclusions: This novel GFP-based microplate assay combines sensitivity and rapidity, is easily amenable to HTS Setups, making it an assay of choice for cytotoxicity evaluation. (C) 2001 Wiley Liss, Inc.

L6 ANSWER 11 OF 25 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
2001095696 EMBASE Interactions between human osteoarthritic chondrocytes and synovial fibroblasts in co-culture. Huch K.; Stove J.; Gunther K.P.; Puhl W.. Dr. K. Huch, Department of Orthopaedic Surgery, University of Ulm, Oberer Eselsberg 45, 89081 Ulm, Germany. klaus.huch@medizin.uni-ulm.de. Clinical and Experimental Rheumatology 19/1 (27-33) 2001.
Refs: 38.
ISSN: 0392-856X. CODEN: CERHDP. Pub. Country: Italy. Language: English. Summary Language: English.
AB Objective. To imitate the in vivo joint situation and to allow cell interactions, a co-culture system of human osteoarthritic chondrocytes and synovial fibroblasts from a single joint was established and characterized with or without stimulation by IL-1β. Methods. Culture settings included chondrocytes in alginate alone, synovial fibroblasts in monolayer alone and a co-culture of both. Proteoglycan (PG) synthesis was measured by (35)S-incorporation, PG content by a dimethylmethylene blue assay, DNA content by a **fluorometric assay**, and prostaglandin-E(2) and IL-1β release by ELISA. Results. In co-culture PG synthesis by chondrocytes was significantly reduced in the presence of IL-1β (1 ng/ml) compared to controls. PG content of chondrocyte cultures was reduced for controls and IL-1β treated co-cultures. Synovial fibroblasts in co-culture did not show significant change of PG synthesis or content when compared to cells in mono-cell culture. PG release into the medium was relatively high in co-cultures. IL-1β significantly decreased the proliferation rate of chondrocytes in co-cultures and slightly increased prostaglandin-E(2) release. Conclusions. Co-culturing of osteoarthritic chondrocytes and synovial fibroblasts from a single human joint allows interactions between both entities and may offer a useful tool to study the effects of mediators or new drugs under more in vivo like conditions compared to mono-cell cultures.

L6 ANSWER 12 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN
2000:861787 Document No. 134:38853 A **fluorometric assay** for caspase activity that uses fusion proteins of green fluorescent protein that exhibit fluorescent resonance energy transfer. Xanthoudakis, Steven; Tawa, Paul; Cassady, Robin; Nicholson, Donald (Merck Frosst Canada and Co., Can.). PCT Int. Appl. WO 2000073437 A1 20001207, 50 pp. DESIGNATED STATES: W: AU, CA, JP, US, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-CA620 20000525. PRIORITY: US 1999-PV136286 19990527.
AB Provided are substrates and assays for the identification of caspase activators and inhibitors. The substrates are fusion proteins comprising two green fluorescent proteins (GFPs) with a linker peptide comprising at

least one caspase cleavage site. The intact fusion protein exhibits fluorescent resonance energy transfer (FRET) between the GFPs. Following caspase cleavage of the linker peptide, the two GFPs become separated and FRET is diminished. The method can be adapted to high throughput assays. Sequences claimed in the document were not published with it.

L6 ANSWER 13 OF 25 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 3

2001116798 EMBASE Fluorometric screening for metabolism-based drug-drug interactions. Crespi C.L.; Stresser D.M.. C.L. Crespi, GENTEST Corporation, 6 Henshaw Street, Woburn, MA 01801, United States. crespi@gentest.com. Journal of Pharmacological and Toxicological Methods 44/1 (325-331) 2000.
Refs: 26.
ISSN: 1056-8719. CODEN: JPTMEZ.
Publisher Ident.: S 1056-8719(00)00112-X. Pub. Country: United States.
Language: English. Summary Language: English.

AB Inhibition of cytochromes P-450 (CYP) is a principal mechanism for metabolism-based drug interactions. In vitro methods for quantitatively measuring the extent of CYP inhibition are well-established. Classical methods use drug molecules as substrates and HPLC-based analysis. However, methodologies, which do not require HPLC separations for data acquisition generally offer higher throughputs and lower costs. Multiwell plate-based, direct, **fluorometric assays** for the activities of the five principal drug-metabolizing enzymes are available and parameters for the use of these substrates to measure CYP inhibition have been established. This methodology is quantitative, rapid, reproducible, and compatible with common high throughput screening instrumentation. This article describes approaches to establishing this methodology in a drug-discovery support program. .COPYRGT. 2001 Elsevier Science Inc.

L6 ANSWER 14 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN

2000:850763 Document No. 135:45 Fluorometric high-throughput screening for inhibitors of cytochrome P450. Miller, Vaughn P.; Stresser, David M.; Blanchard, Andrew P.; Turner, Stephanie; Crespi, Charles L. (Gentest Corporation, Woburn, MA, 01801, USA). Annals of the New York Academy of Sciences, 919(Toxicology for the Next Millennium), 26-32 (English) 2000. CODEN: ANYAA9. ISSN: 0077-8923. Publisher: New York Academy of Sciences.

AB A review, with 21 refs. Rapid screening for cytochrome P 450 inhibitors is part of the current paradigm for avoiding development of drugs likely to give clin. pharmacokinetic drug-drug interactions and associated toxicities. The authors have developed microtiter plate-based, direct, **fluorometric assays** for the activities of the principal human drug-metabolizing enzymes, CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4, as well as for CYP2A6, which is an important enzyme in environmental toxicol. These assays are rapid and compatible with existing high-throughput assay instrumentation. For CYP1A2, CYP2C8, CYP2C9, CYP2C19, and CYP2D6, the potency of enzyme inhibition (IC50) is consistent regardless of the probe substrate or assay method employed. In contrast, CYP3A4 inhibition for an individual inhibitor shows significant differences in potency (>300-fold) depending on the probe substrate being used. The authors have investigated these differences through the use of several structurally distinct fluorescent substrates for CYP3A4 and several classical substrate probes (e.g., testosterone, nifedipine, and midazolam), with a panel of known, clin. significant, CYP3A4 inhibitors. The use of multiple probe substrates appears to be needed to characterize the inhibition potential of xenobiotics for CYP3A4.

L6 ANSWER 15 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN

1999:83700 Document No. 130:263863 Fully automated analysis of activities catalyzed by the major human liver cytochrome P450 (CYP) enzymes: assessment of human CYP inhibition potential. Moody, G. C.; Griffin, S.

J.; Mather, A. N.; McGinnity, D. F.; Riley, R. J. (Department of Physical and Metabolic Sciences, Loughborough, LE11 5RH, UK). Xenobiotica, 29(1), 53-75 (English) 1999. CODEN: XENOBH. ISSN: 0049-8254. Publisher: Taylor & Francis Ltd..

AB 1. Fully automated inhibition screens for the major human hepatic cytochrome P 450s have been developed and validated. Probe assays were the fluorometric-based ethoxyresorufin O-deethylation for CYP1A2 and radiometric anal. of erythromycin N-demethylation for CYP3A4, dextromethorphan O-demethylation for CYP2D6, naproxen O-demethylation for CYP2C9 and diazepam N-demethylation for CYP2C19. For the radiometric assays > 99.7% of 14C-labeled substrate was routinely extracted from incubations by solid-phase extraction 2. Furafylline, sulfaphenazole, omeprazole, quinidine and ketoconazole were identified as specific markers for the resp. CYP1A2 (IC50 = 6 μM), CYP2C9 (0.7 μM), CYP2C19 (6 μM), CYP2D6 (0.02 μM) and CYP3A4 (0.2 μM) inhibition screens. 3. For the radiometric methods, a two-point IC50 estimate was validated by correlating the IC50 obtained with a full (seven-point) assay (R2 = 0.98, $p < 0.001$). The two-point IC50 estimate is useful for initial screening, while the full IC50 method provides more definitive quantitation, where required. 4. IC50 determined for a series of test compds. in human liver microsomes and cytochrome P 450 cDNA-expressed enzymes were similar (R2 = 0.89, $p < 0.001$). In particular, the CYP2D6, CYP2D6 and CYP3A4 screens demonstrated the flexibility to accept either enzyme source. As a result of incomplete substrate selectivity, expressed enzymes were utilized for anal. of CYP2C9 and CYP2C19 inhibition. Good agreement was demonstrated between IC50 determined in these assays to IC50 published by other labs. using a wide range of anal. techniques, which provided confidence in the universality of these inhibition screens. 5. These automated screens for initial assessment of P 450 inhibition potential allow rapid determination of IC50. The radiometric assays are flexible, sensitive, robust and free from anal. interference, and they should permit the identification and eradication of inhibitory structural motifs within a series of potential drug candidates.

L6 ANSWER 16 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN

2000:790102 Document No. 134:304873 Time-resolved **fluorometric assays**. Hemmila, Ilkka; Kovanen, Satu (Wallac Oy, UK). Innovations in Pharmaceutical Technology, 99(3), 42-44, 46-49 (English) 1999. CODEN: IPTNBO. Publisher: Chancery Media Ltd..

AB A review with 2 refs. time-resolved **fluorometric assays** in drug discovery. Topics discussed include the lanthanide chelate labels, time-resolved fluorometric imaging, homogeneous assays, and instrumentation.

L6 ANSWER 17 OF 25 MEDLINE on STN DUPLICATE 4

1999086073 Document Number: 99086073. PubMed ID: 9870526. A **fluorometric assay** for the measurement of endothelial cell density in vitro. Parandoosh Z; Bogowitz C A; Nova M P. (IRORI, La Jolla, California 92037-1030, USA.. zahra@san.vr.com) . IN VITRO CELLULAR AND DEVELOPMENTAL BIOLOGY. ANIMAL, (1998 Nov-Dec) 34 (10) 772-6. Journal code: 9418515. ISSN: 1071-2690. Pub. country: United States. Language: English.

AB A **fluorometric assay** for determining endothelial cell numbers based on the endogenous enzyme acid phosphatase is described. In preliminary studies, three substrates--p-nitrophenyl phosphate, 4-methylumbelliferyl phosphate, and 2'-[2-benzthiazoyl]-6'-hydroxy-benthiazole phosphate (AttoPhos)--were compared with respect to their kinetic, optimum assay conditions, sensitivity, and detection limits. Only AttoPhos was found to have a high degree of sensitivity, reliability, and reproducibility for measuring both high and low cell numbers in the same plate. In subsequent experiments, assay conditions were validated for measuring endothelial cell density in response to basic fibroblast

growth factor and fumagillin. Furthermore, the AttoPhos assay revealed a linear correlation between acid phosphatase activity and cell number in many cell types, including BALB/3T3, CHO-K1, A431, MCF7, 2008, SK-OV-3, T47-D, and OVCAR-3. This assay is potentially valuable for use in many in vitro systems in which the quantitation of cell density and proliferation is necessary. The practical advantages of AttoPhos assay for measuring endothelial cell numbers include (1) nonradioactivity, (2) simplicity, (3) economy, (4) speed of assessment of proliferation of large number of samples, and (5) amenability to high-throughput **drug screening**.

L6 ANSWER 18 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN
1998:515293 Document No. 129:239396 Dialysis tubing implant assay in the rat: a novel in vivo method for identifying inhibitors of matrix metalloproteinases. Dijoseph, John F.; Sharr, Michele A. (Wyeth-Ayerst Research, Princeton, NJ, 08543-4000, USA). Drug Development Research, 43(4), 200-205 (English) 1998. CODEN: DDREDK. ISSN: 0272-4391. Publisher: Wiley-Liss, Inc..

AB A novel model to assess the in vivo bioactivity of matrix metalloproteinase (MMP) inhibitors is described. A solution containing the target enzyme, either stromelysin (MMP-3) or collagenase (MMP-1), was put inside dialysis tubing which was then implanted either i.p. or s.c. in the back of a rat. The rat was dosed with inhibitor, the dialysis tubing was recovered after a set period of time, and its contents were analyzed for inhibition of enzyme activity. The MMP inhibitors tested were each able to significantly block enzyme activity after either i.v. or oral administration. In vitro, a min. of 30 min was required for the drugs to maximally equilibrate across the dialysis membrane and inhibit enzyme activity. In the rat, significant inhibition of stromelysin activity was observed at 45 min after i.v. drug administration but was maximal at 60 min. Dialysis tubing of various mol. weight cutoffs from 3,500 to 14,000 did not affect the amount of inhibition exerted by the compds. The enzyme activity recovered from the dialysis tubing was quantitated by either spectrophotometric or **fluorometric assays** using specific substrates. This model determined the bioactivity of different chemical classes of known inhibitors of stromelysin and collagenase in a rapid and convenient manner. The dialysis tubing implant model can be expanded to assess the pharmacodynamic bioactivity of a diverse group of drugs using various targets inside the dialysis tubing.

L6 ANSWER 19 OF 25 MEDLINE on STN DUPLICATE 5
93046178 Document Number: 93046178. PubMed ID: 1423302. Comparison of mouse and human colon tumors with regard to phase I and phase II drug-metabolizing enzyme systems. Massaad L; de Waziers I; Ribrag V; Janot F; Beaune P H; Morizet J; Gouyette A; Chabot G G. (Laboratoire de Pharmacologie Clinique, Institut Gustave-Roussy, (INSERM U 140 and CNRS URA 147), Villejuif, France.) CANCER RESEARCH, (1992 Dec 1) 52 (23) 6567-75. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB Since human colorectal tumors are insensitive to most chemotherapeutic agents, there is a need for the discovery of new drugs that would show activity against this disease. In an attempt to better appreciate the relevance of a widely used mouse colon tumor (colon adenocarcinoma Co38) as a screening model for human colorectal tumors, we compared the main phase I and phase II drug-metabolizing enzyme systems in both tumoral and nontumoral colon tissues. The following enzymes were assayed by Western blot: cytochromes P-450 (1A1/A2, 2B1/B2, 2C, 2E1, and 3A), epoxide hydrolase, and glutathione-S-transferases (GST-alpha, -mu, and -pi). The activities of the following enzymes or cofactors were determined by spectrophotometric or **fluorometric assays**: total cytochrome P-450, 1-chloro-2,4-dinitrobenzene-GST, selenium-independent

glutathione peroxidase, 3,4-dichloronitrobenzene-GST, ethacrynic acid-GST, total glutathione, epoxide hydrolase, UDP-glucuronosyltransferase, beta-glucuronidase, sulfotransferase, and sulfatase. Results obtained by Western blot showed that mouse colon adenocarcinoma Co38 did not express any of the probed cytochromes P-450, whereas human colorectal tumors expressed only low levels of cytochrome P-450 3A. GST-alpha and GST-pi were detected in all tumoral and nontumoral tissues of both species. The neutral GST-mu was expressed in all murine tissues investigated and was found to be polymorphic in human tissues. For human peritumoral and tumoral colorectal tissues there was no significant difference between GST isoenzyme levels, whereas mouse colon adenocarcinoma Co38 had a lower expression of GST-mu and GST-pi, compared to normal mouse colon. Enzymatic activities for glutathione peroxidase, 3,4-dichloronitrobenzene-GST, and ethacrynic acid-GST confirmed the Western blot results for GST-alpha, GST-mu, and GST-pi, respectively. Total GSH levels were similar between murine and human tumors but were 3-fold higher in human tumors than in peritumoral tissues, whereas they were 7-fold lower in mouse colon tumor Co38, compared to normal mouse colon. Epoxide hydrolase was not expressed in either mouse colon adenocarcinoma Co38 or normal mouse colon tissues, whereas it was expressed in human colon peritumoral and tumoral tissues at similar levels. No significant difference was observed between human tumors and peritumoral tissues for UDP-glucuronosyltransferase, beta-glucuronidase, sulfotransferase, and sulfatase. For murine colon tissues, the conjugation pathways (UDP-glucuronosyltransferase and sulfotransferase) were lower in colon adenocarcinoma Co38, whereas the converse was observed for the corresponding hydrolytic enzymes (beta-glucuronidase and sulfatase).(ABSTRACT TRUNCATED AT 400 WORDS)

L6 ANSWER 20 OF 25 MEDLINE on STN

92379484 Document Number: 92379484. PubMed ID: 1726673. A fluorometric rapid microassay to identify anti-proliferative compounds for human melanoma cells in vitro. Zouboulis C C; Garbe C; Krasagakis K; Kruger S; Orfanos C E. (Department of Dermatology, Free University of Berlin, Germany.) MELANOMA RESEARCH, (1991 Jun-Jul) 1 (2) 91-5. Journal code: 9109623. ISSN: 0960-8931. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A simple, rapid and reproducible assay for the determination of melanoma cell proliferation in vitro is described, based on the hydrolysis of a fluorogenic substrate by cell esterases in the cytoplasm of living cells. Human melanoma cells were cultured at several densities in 96-well culture plates for 24 h and were then incubated with 4-methylumbelliferyl heptanoate. The generated fluorescence showed a strong correlation with the cell numbers, similar to those assessed by determining the [3H]thymidine incorporation into cellular DNA and by quantifying the fluorescence obtained after DNA labelling with Hoechst 33258. The latter, however, was less sensitive and exhibited higher standard deviations. In addition, the method reliably detected the anti-proliferative effects of the anti-cancer compounds cisplatin and vindesine. It is, therefore, suggested that the **fluorometric assay** with 4-methylumbelliferyl heptanoate as substrate could prove useful for the screening of potential anti-cancer agents with anti-proliferative activity.

L6 ANSWER 21 OF 25 MEDLINE on STN

90355549 Document Number: 90355549. PubMed ID: 2388483. Chemotherapeutic drug sensitivity testing of human leukemia cells in vitro using a semiautomated **fluorometric assay**. Larsson R; Nygren P; Ekberg M; Slater L. (Departments of Clinical Pharmacology, University Hospital, Uppsala University, Sweden.) LEUKEMIA, (1990 Aug) 4 (8) 567-71. Journal code: 8704895. ISSN: 0887-6924. Pub. country: United States. Language: English.

AB A simple and reproducible semiautomated fluorometric method for drug sensitivity testing of leukemic cells in microculture is described. The assay is based on hydrolysis of nonfluorescent fluorescein diacetate (FDA) to a strongly fluorescent product (fluorescein) by cells with intact plasma membranes after 72 hr of culture and was in the present study applied to acute lymphocytic leukemia (ALL) cell lines and specimens from patients with lymphocytic and myelocytic leukemia. FDA fluorescence was linearly related to viable cell number within a wide range of cell densities (3-4 logs) as well as in the presence of different added proportions of dead cells. The assay reliably detects high and low grade resistance to vincristine (vcr) and daunorubicin, respectively, as well as the subsequent reversal of vcr resistance by cyclosporin A and the calcium channel blocker verapamil. Using ALL cell lines, drug sensitivity was in good correspondence with data obtained by the microculture tetrazolium assay. Furthermore, drug sensitivity data of fresh leukemia cells from patients with leukemia were readily obtained. The results indicate that the presently described method is applicable for simple and reliable chemosensitivity testing of leukemia cell lines as well as tumor specimens from patients with leukemia.

L6 ANSWER 22 OF 25 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

79232861 EMBASE Document No.: 1979232861. Low cerebrospinal fluid γ-aminobutyric acid content in seizure patients. Wood J.H.; Hare T.A.; Glaeser B.S.; et al.. Surg. Neurol. Branch, Nat. Inst. Neurol. Communicat. Disorders Stroke, NIH, Bethesda, Md., United States. Neurology 29/9 I (1203-1208) 1979.
CODEN: NEURAI. Pub. Country: United States. Language: English.

AB γ-Aminobutyric acid (GABA) has been implicated in the neurochemistry of epilepsy. Lumbar cerebrospinal fluid (CSF) GABA concentrations determined using an ion-exchange **fluorometric assay** reflect brain GABA content. The mean lumbar CSF GABA concentration among 21 medicated patients with intractable seizures was significantly lower ($p < 0.001$) than that of 20 unmedicated normal volunteers. Patients with generalized tonic-clonic (grand mal) and complex partial (psychomotor) seizures had significantly lower ($p < 0.05$) CSF GABA concentrations than those with simple partial (focal sensory/mortor) seizures. Although lumbar CSF GABA levels in our seizure patients did not significantly correlate with serum concentrations of phenytoin, phenobarbital, or primidone, additional study of medication-free epileptic patients may be required to evaluate the possibility of anticonvulsant-drug-induced CSF GABA alterations.

L6 ANSWER 23 OF 25 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

79142531 EMBASE Document No.: 1979142531. Modes of regional chemotherapy. Karakousis C.P.; Kanter P.M.; Lopez R.; et al.. Dept. Surg. Oncol., Roswell Park Mem. Inst., Buffalo, N.Y. 14263, United States. Journal of Surgical Research 26/2 (134-141) 1979.
CODEN: JSGRA2. Pub. Country: United States. Language: English.

AB Injection of Am (2 mg/kg) into the hepatic artery or portal vein of dogs given higher liver tissue levels (15.1 μg/g) than systemic intravenous administration (6.4 μg/g). This difference is increased by injection of the drug distal to the temporally occluded hepatic artery or portal vein. Bolus injection of Am (1 mg/kg) into the femoral artery of dogs results in no detectable ipsilateral calf muscle levels and no subsequent macroscopic changes in the extremity. When injected distally to the temporarily occluded femoral artery, muscle drug levels were below the level of sensitivity of the **fluorometric assay** used, but all animals showed varying degrees of ipsilateral skin ulcerations and melanosis. With a tight tourniquet applied to the extremity proximally and maintained for 15 min after Am injection, 40-fold or higher muscle levels

of Am were found, compared to those after simple intraarterial injection. There was fairly uniform drug distribution throughout the infused limb and minimal systemic leak. Severe ulcerations occurred after injection of 1 mg Am/kg with tourniquet application. With reduction of the dose to 0.25 and 0.125 mg/kg, erythema in the first 2 weeks and melanosis of the skin at 2 to 4 weeks occurred and involved the whole extremity. This technique of intraarterial injection of chemotherapeutic drug(s) with a proximal tourniquet has a potential clinical applicability in extremity tumors; high tissue drug levels are obtained and frequent administration is possible via an indwelling catheter. With the present development of the ability to assay microconcentrations of drugs in tissues, a critical reappraisal of the methods of regional infusion chemotherapy should now be possible, in association with the physiochemical characteristics of the drugs used.

L6 ANSWER 24 OF 25 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
1979:216500 Document No.: PREV197968019004; BA68:19004. EFFECTS OF ANTI HYPERTENSIVE DRUGS ON HEPATIC HEME BIOSYNTHESIS AND EVALUATION OF FERRO CHELATASE INHIBITORS TO SIMPLIFY TESTING OF DRUGS FOR HEME PATHWAY INDUCTION. ANDERSON K E [Reprint author]. ROCKEFELLER UNIV, NEW YORK, NY 10021, USA. Biochimica et Biophysica Acta, (1978) Vol. 543, No. 3, pp. 313-327.
CODEN: BBACAQ. ISSN: 0006-3002. Language: ENGLISH.
AB Effects of a series of antihypertensive drugs on the activity of δ-aminolevulinate synthase and on the formation of porphyrins and cytochrome P-450 were examined in the 18 day old chick embryo liver in ovo. Hydralazine, pargyline, phenoxybenzamine, clonidine and spironolactone induced δ-aminolevulinate synthase in this system. These drugs therefore have the potential to precipitate clinical expression in human hereditary hepatic porphyrias and should be avoided or used with caution in patients with these disorders. Differential effects of these and other drugs were observed in the avian liver, in that δ-aminolevulinate synthase was more commonly induced than were porphyrins and cytochrome P-450; the synthase was usually highest 6-12 h after injection, but porphyrins and cytochrome P-450 were highest at 24 h. Marked porphyrin accumulation was not seen with many drugs that induce δ-aminolevulinate synthase and cytochrome P-450 but was more characteristic of compounds that reduced the metabolism of protoporphyrin to heme, such as 1,4-dihydro-3,5-dicarbethoxycollidine (DDC) and high doses of hydralazine. A sensitive and convenient method to test for capacity to induce heme biosynthesis was adapted for use in the chick embryo liver. This employed a relatively small priming dose (0.25 mg) of DDC given with a drug being tested and a **fluorometric assay** of porphyrins in a liver homogenate obtained at 24 h. This simple method should facilitate screening for those drugs which induce the synthesis of δ-aminolevulinate synthase and/or cytochrome P-450 and are potentially dangerous to patients with hereditary hepatic porphyria.

L6 ANSWER 25 OF 25 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
74195233 EMBASE Document No.: 1974195233. Improved **fluorometric assay** for digoxin to determine tablet dissolution. Borst S.I.. Dept. Drugs Therapeut., Ontario Min. Hlth, Toronto, Canada. Canadian Journal of Pharmaceutical Sciences 9/1 (27-29) 1974.
CODEN: CNJPAZ. Language: English.

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20 FEB 2004
L1 6355 S FLUOROMETRIC ASSAY
L2 139 S L1 AND CASPASE
L3 0 S L2 AND FLUOROCENT RATIO
L4 45 DUP REMOVE L2 (94 DUPLICATES REMOVED)
L5 36 S L1 AND DRUG SCREENING
L6 25 DUP REMOVE L5 (11 DUPLICATES REMOVED)

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L7 299 L1 AND RATIO

=> s l7 and fluoresence
L8 0 L7 AND FLUORESENCE

=> s l7 and peptide substrate
L9 5 L7 AND PEPTIDE SUBSTRATE

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L10 3 DUP REMOVE L9 (2 DUPLICATES REMOVED)

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L10 ANSWER 1 OF 3 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
2003:149056 The Genuine Article (R) Number: 640VW. Development of intramolecularly quenched fluorescent peptides as substrates of angiotensin-converting enzyme 2. Yan Z H; Ren K J; Wang Y F; Chen S; Brock T A; Rege A A (Reprint). Texas Biotechnol Corp, Dept Pharmacol, 7000 Fannin St, Houston, TX 77030 USA (Reprint); Texas Biotechnol Corp, Dept Pharmacol, Houston, TX 77030 USA; Texas Biotechnol Corp, Dept Med Chem, Houston, TX 77030 USA. ANALYTICAL BIOCHEMISTRY (15 JAN 2003) Vol. 312, No. 2, pp. 141-147. Publisher: ACADEMIC PRESS INC ELSEVIER SCIENCE. 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA. ISSN: 0003-2697. Pub. country: USA . Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Angiotensin-converting enzyme 2 (ACE2 or ACEH) is a novel angiotensin-converting enzyme-related carboxypeptidase that cleaves a single amino acid from angiotensin 1, des-Arg bradykinin, and many other bioactive peptides. Using des-Arg bradykinin as a template, we designed a series of intramolecularly quenched fluorogenic **peptide substrates** for ACE2. The general structure of the substrates was F-X-Q, in which F was the fluorescent group, Abz, Q was the quenching group (either Phe(NO2) or Tyr(NO2)), and X was the intervening peptide. These substrates were selectively cleaved by recombinant human ACE2, as shown by MS and HPLC. Quenching efficiency increased as the peptide sequence was shortened from 8 to 3 aa, and also when Tyr(NO2) was used as a quenching group instead of Phe(NO2). Two of the optimized substrates, TBC5180 and TBC5182, produced a signal:noise **ratio** of better than 20 when hydrolyzed by ACE2. Kinetic measurements with ACE2 were as follows: TBC5180, K-m = 58 muM and k(cat)/K-m = 1.3 x 10(5) M-1 s(-1); TBC5182, K-m = 23 muM and k(cat)/K-m = 3.5 x 10(4) M-1 s(-1). Thus, based on hydrolysis rate, TBC5180 was a better substrate than TBC5182. However, TBC5180 was also hydrolyzed by ACE, whereas TBC5182 was not cleaved, suggesting that TBC5182 was a selective for ACE2. We conclude that these two peptides can be used as fluorescent substrates for high-throughput screening for selective inhibitors of ACE2 enzyme. (C) 2003 Elsevier Science (USA). All rights reserved.

L10 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2004 ACS on STN
1993:466308 Document No. 119:66308 A fluorescent **peptide substrate** for the surface metalloprotease of Leishmania. Bouvier, Jacques; Schneider, Pascal; Malcolm, Bruce (Sch. Med., Univ. California,

San Francisco, CA, 94143, USA). Experimental Parasitology, 76(2), 146-55 (English) 1993. CODEN: EXPAAA. ISSN: 0014-4894.

AB A fluorescent oligopeptide substrate for the promastigote surface protease (PSP) of Leishmania was designed using the data reported for the substrate specificity of the enzyme (Bouvier, J. et al., 1990). The indole fluorescence of the tryptophan residue was efficiently quenched through resonance energy transfer by an N-terminal dansyl group located 5 amino acid residues away. The heptapeptide, dansyl-A-Y-L-K-K-W-V-NH2, was cleaved by PSP between the tyrosine and leucine residues with a kcat/Km **ratio** of 8.8 + 106 M-1 sec-1. Hydrolysis by the enzyme results in a time-dependent increase of fluorescence intensity of 3.7-fold. Assays can be designed based on the tryptophan fluorescence at 360 nm or by individual product analyses using thin-layer chromatog. The synthetic substrate is readily cleaved by the metalloprotease at the surface of fixed promastigotes. The specificity and sensitivity of such internally quenched fluorescent **peptide substrate** will facilitate the identification of novel inhibitors for the enzyme and aid in detailed studies on its enzymol.

L10 ANSWER 3 OF 3 EMBASE
on STN DUPLICATE 1

82201223 EMBASE Document No.: 1982201223. A rapid **fluorometric assay** for measurement of peptidase activity. Porter D.H.; Swaisgood H.E.; Catignani G.L.. Dep. Food Sci. Biochem., North Carolina State Univ., Raleigh, NC 27650, United States. Analytical Biochemistry 123/1 (41-48) 1982.
CODEN: ANBCA2. Pub. Country: United States. Language: English.

AB A rapid method using orthophthaldialdehyde and mercaptoethanol to form a fluorescent adduct with released α-amino groups was developed and characterized as a means for assaying peptidase activity on physiological **peptide substrates**. A typical assay consisted of withdrawing a 10-μl aliquot of the substrate-enzyme solution at various time intervals, which was then added to 3 ml of reagent; the fluorescence was measured relative to 0.1 μg/ml quinine sulfate in 0.1 N H2SO4 following a standardized 2-min reaction time. The assay can be performed either in the presence or absence of sodium dodecyl sulfate; addition of the detergent can be a convenient means for stopping the reaction. Contrary to previous suggestions, addition of detergent was found to slightly decrease fluorescence of dipeptide adducts with this reagent rather than causing a substantial enhancement. However, enhancement was observed for cases where the spacing between isoindoles or between isoindole and indole was similar to that for the disubstituted derivative of lysine. This method was characterized by assaying porcine intestinal peptidase on the peptides Leu-Gly, Gly-Gly, Ala-Gly, and Gly-Leu-Tyr. Using an immobilized form of this enzyme, excellent agreement was found between amino acid determinations and fluorescence-derived calculated values for the fraction of bonds hydrolyzed for dipeptide substrates. It was shown that the fluorescence **ratio** (F(t)-F0)/(F(c)-F0) corresponds to the fractional hydrolysis of dipeptides where F(t), F0, and F(c) are the relative fluorescences at time t, time zero, and complete hydrolysis, respectively.